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HISTOCOMPATIBILITY GENETICS OF BULLFROG POPULATIONS[1]

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Received August 5, 1960

Genetic studies of anuran species have been limited for the most part by failure of the F_1 generation to reach maturity under laboratory conditions. Thus data on backcross and F_2 progenies are not available and the mode of inheritance even of overt traits such as pigment patterns remains problematical (Anderson and Volpe, 1958). Analyses of natural populations have revealed much phenotypic variation in morphological and pigmentary characteristics. However, most of these variants are of the graded, quantitative type which are often influenced by age and environment (see Volpe, 1957). Studies of population genetics and dynamics would be facilitated by a technique enabling the investigator to compare the frequencies of clear-cut genetic characters such as cellular antigens. This approach is available in the skin homograft reaction, which is known to depend upon a large number of gene-determined, cellular isoantigens (Hildemann and Owen, 1956; Barnes and Krohn, 1957).

Experience with many vertebrates supports the generalization that a graft will soon fail if it contains a gene controlling the production of a potent antigen different from any in the host (see review by Owen, 1959). Accelerated rejection of a subsequent skin graft from the same donor is the rule, and reveals the immunogenetic basis of the incompatibility. If the second graft is from a different donor, it may or may not evoke the "second-set" accelerated reaction, depending on whether the two donors share one or more strong histocompatibility antigens. Antigens or alleles possessed only by the host and absent from the donor's tissues apparently have no influence on the outcome of the grafting. A test grafting procedure employing two different donors and a recipient chosen at random was used in the present study.

A previous investigation of the bullfrog (Hildemann and Haas, 1959) showed that from larval stage 25 (~ 60 days of age) on, this species regularly destroys skin homografts by a sensitive isoimmune response that is closely temperature dependent. The capacity to reject skin homografts, once developed, remains essentially unchanged whatever the age or stage of development, despite the profound physiologic changes that occur during metamorphosis. At 25° C the median survival time (MST) of first-set homografts in a series of 177 random tadpoles at standard stages 25–29 from Crystal Lake, Califor-

[1] This investigation was supported by Research Grant C-4027 to the senior author from the National Cancer Institute, U. S. Public Health Service.

nia, ranged from 11.8 to 13.6 days, while second-set grafts (i.e. repeat grafts from the same donors after destruction of initial first-set grafts) had an MST of only 5.1 days. The earliest first-set rejections recorded were at 9 days, while the latest second-set rejections occurred at 7 days. Thus there was no overlap in the survival end-points of skin grafts in the different sets. The design of the experiments to be described was therefore based on the assumption that accelerated rejection (in 7 days or less) of a second graft from a different donor would indicate that the two donors shared at least one strong histocompatibility antigen.

Material and Methods

Three geographically isolated populations of bullfrog (*Rana catesbeiana*) tadpoles from Southern California were investigated. All the tadpoles employed were at advanced stages of development (standard stages 25–29), known to be immunologically mature. Crystal Lake supports a large bullfrog population, evidenced by the presence of at least several thousand tadpoles throughout the year. The Malibu Lake population appeared to be relatively small, with a few hundred tadpoles at most, even in late summer. The Brescia population sample (Brescia Frog Farm) was taken from a single pond. In contrast with the Crystal Lake population each of the latter samples consisted of tadpoles of similar size and at stage 25; hence, many of these individuals could well have been siblings.

In all experiments each tadpole was marked by a numbered tag inserted through the tail. The animals were maintained in aerated, filtered $36'' \times 16'' \times 16''$ aquariums and were well fed on a diet consisting of mixed cereals, beef liver, and aquatic plants. The water temperature was thermostatically controlled at $25 \pm 0.5°$ C, and all animals were acclimatized for at least one week before grafting. Single skin homografts were exchanged between animals in pairs and subsequently scored for time of rejection. For later test grafting the pair combinations were simply changed. Thus each tadpole served as both donor and recipient of two successive homografts, a procedure that makes maximal use of animals and facilities. The techniques employed and the scoring of graft survival end-points *in vivo* are described in detail by Hildemann and Haas (1959). Disintegration of the evenly distributed and conspicuous corolla-type xanthophores in skin homografts provided the best available survival end-point in this species. Identical control autografts were uniformly and permanently successful.

We are indebted to Professor Ray D. Owen, Division of Biology, California Institute of Technology, for helpful discussions of the data presented.

Experimental Results

Reciprocal skin homografts were exchanged between 38 tadpoles in randomly selected pairs collected from Crystal Lake. All grafts were rejected after typical first-set reactions between 9 and 16 days. Second (test) homografts were made in different donor–recipient combinations after 20–21 days. Initial homografts were always placed on the median-dorsal aspect of the body just posterior to the eyes, while test homografts were transplanted about 1 cm posterior to the site of the first homograft. Only 4 of the 38 test grafts were destroyed within 7 days. Thus 10.5 per cent of random pairs of Crystal Lake tadpole donors shared one or more histocompatibility antigens not present in the recipients. When 24 Crystal Lake siblings hatched from a single egg mass and reared in the laboratory were similarly tested, 14 tadpoles showed accelerated rejection of their test homografts. This high frequency of antigen sharing (58.3 per cent) among sibling donors relative to sibling recipients is in marked contrast to that found in the random sample of the same large population. This observed difference is highly significant ($p < .0001$).

When 36 tadpoles from Malibu Lake

were randomly test-grafted in the same manner, 18 or exactly 50 per cent had a curtailed graft survival time of 7 days or less. Similarly, 38 out of 83 tadpoles (45.8 per cent) from the Brescia pond population rapidly destroyed their test homografts after typical second-set reactions. The probability is 0.688 that the observed difference could have occurred by chance in random sampling from the same population. By contrast, the probability is only .0001 and .0002 that the proportion of accelerated rejections in the Brescia and Malibu samples could have been observed in the Crystal Lake population. The time interval between the initial and test homograft exchanges was 18–21 days in all instances. The quantitative results are summarized in Table 1.

Although it was surprising to find a frequency of antigen sharing in the Malibu Lake and Brescia Pond donors almost as great as that observed in Crystal Lake siblings, it is probable that the smallness of the former populations led to the inadvertent inclusion of many siblings in the experiments. This supposition is also supported by the finding that four first-set grafts and one test graft between Brescia tadpoles survived for 21 to 25 days. Four first-set grafts between Crystal Lake siblings similarly showed slightly, but significantly prolonged survival at 25° C. The 5 to 6 fold increase in common histocompatibility antigens among Crystal Lake sibling donors as opposed to random donors was striking, and suggests that many histocompatibility alleles at various loci are segregating in the wild population. The use of successive sib donors with unrelated recipients might provide a more direct measure of antigen-sharing among such donors. To what extent the percentage of test homografts showing accelerated rejection may be accepted as an index of genetic diversity must await further population studies. So far we have studied some 573 skin homografts involving normal, immunologically mature tadpoles in different donor–recipient combinations. Since none of these grafts was compatible, although many pairs of siblings were tested, the histocompatibility gene diversity would appear to be as great in this species as in any other studied to date. Obviously, no simple genetic model will account for the failure to find compatibility in any of more than 500 random combinations. In this connection, the alleles and antigens involved are not necessarily in a one-to-one relation. Studies of red cell immunogenetics in various mammals and birds clearly indicate that more than one locus may specify an antigen and a

TABLE 1. *Survival time data on second (test) homografts to larval recipients that had previously rejected a graft from a different larval donor*

Population Source*	Number of recipients receiving test homografts	Time interval (days) between initial and test homograft	Number of test homografts surviving 7 days or less (accelerated rejection)	Per cent of test homografts showing accelerated rejection	Probability that observed difference could have occurred by chance in random sampling**
a. Crystal Lake (Random)	38	20–21	4	10.5	(a – b) $< .0001$
b. Crystal Lake (Siblings)	24	21	14	58.3	(a – c) .0002
c. Malibu Lake (Random)	36	19	18	50.0	(a – d) .0001
d. Brescia Pond (Random)	83	18–21	38	45.8	(c – d) .688

* Three geographically isolated populations from Southern California are indicated. The Crystal Lake siblings were hatched from a single egg mass and reared in the laboratory.

**Significance of difference between pairs of samples (a, b, c, and d) calculated from normal curve table employing the relative deviate.

given locus may affect more than one antigenic molecule (Owen, 1958).

The accelerated reactions indicative of previous immunization were qualitatively distinguishable from those of the first-set type. Thus rapid homograft breakdown was associated with white grafts showing no blood supply or with early hemorrhagic inflammation in the zone of contact with host tissue. In either case, restoration of capillary circulation in the graft was not observed. That skin homografts exchanged between unrelated individuals failed to sensitize for a second-set response to different donors in many or most instances, revealed that there must be a high degree of immunological specificity for the particular combination of donor and recipient involved in the original sensitization. In other words, cross-reactions with respect to similar antigens appeared to be notably infrequent. However, the few test grafts that showed only 10 per cent or less survival of xanthophores at 8 days may have reflected some cross-reactivity toward similar antigenic determinants in the respective donors. Results consistent with the present findings on the specificity of rejection have been reported in skin transplantation experiments with random-bred rabbits (Medawar, 1946), hamsters (Billingham and Hildemann, 1958), and man (Rapaport et al., 1960).

Our results as a whole indicate that *every* tadpole has at least one antigen different from those occurring in any other tadpole. Thus the probability of obtaining a first-set response to an initial homograft is very nearly 1, or 100 per cent. While it might be assumed that intrapopulation proportions of shared antigens (genes) would be higher than those found in interpopulation tests, both types of experiment would have to be done if quantitative, interpopulation comparisons are to be made. Although the immunogenetic approach described lacks genetic precision, it could be useful in population studies like those of Volpe (1957) and others on frogs and of Twitty (1959) on California newts. The same experimental design could be advantageously applied to fish populations as well (Hildemann and Haas, 1960).

Summary

The high degree of specificity of homograft rejection provides a basis for immunogenetic analyses of certain amphibian and fish populations. Three geographically isolated populations of bullfrog tadpoles from Southern California were investigated in the present study. Single skin homografts exchanged between pairs of unrelated animals or siblings at 25° C were found to remain viable for at least 9 days. The pair combinations were changed for subsequent test grafting, so that each tadpole served as both donor and recipient of two successive homografts. Since accelerated rejection within 7 days or less at 25° C invariably occurred when the same donor provided both grafts, it follows that such rejection of a second graft from a different donor would indicate that the two donors shared one or more strong histocompatibility antigens absent from the recipient.

While only 10.5 per cent of random tadpoles from the large Crystal Lake population evinced such shared antigens, 58.3 per cent of known siblings obtained from this population rejected their test homografts by accelerated reactions. This 5 to 6 fold increase in common histocompatibility antigens among siblings is highly significant ($p < .0001$), and indicates that many histocompatibility alleles at various loci must be segregating in the wild population. Similar test grafting of random individuals, including an unknown proportion of siblings taken from two small populations at Malibu Lake and Brescia Pond, revealed 50.0 and 45.8 per cent of shared donor antigens, respectively. Here the probability is 0.688 that the observed difference could have occurred by chance in random sampling from the same population. In contrast, the probability is only .0001 and .0002 that the proportion of accelerated rejections in the Brescia and Malibu

samples could have been observed in the Crystal Lake population.

The finding that all of 573 skin homografts involving tadpoles in different donor–recipient combinations were rejected implies that every tadpole has at least one antigen different from those occurring in any other tadpole. The over-all results are consistent with the assumption that the per cent of antigen sharing shown by test grafting may be regarded as a measure of genetic diversity in population studies.

Literature Cited

Anderson, S. C., and E. P. Volpe. 1958. Burnsi and kandiyohi genes in the leopard frog *Rana pipiens*. Science, **127**: 1048–1049.

Barnes, A. D., and P. L. Krohn. 1957. The estimation of the number of histocompatibility genes controlling the successful transplantation of normal skin in mice. Proc. Roy. Soc. London, (B) **146**: 505–526.

Billingham, R. E., and W. H. Hildemann. 1958. Studies on the immunological responses of hamsters to skin homografts. Proc. Roy. Soc. London, (B) **148**: 216–233.

Hildemann, W. H., and R. Haas. 1959. Homotransplantation immunity and tolerance in the bullfrog. J. Immunol., **83**: 478–485.

——. 1960. Comparative studies of homotransplantation in fishes. J. Cell. Comp. Physiol. (in press).

Hildemann, W. H., and R. D. Owen. 1956. Histocompatibility genetics of scale transplantation. Transpl. Bull., **4**: 132–134.

Medawar, P. B. 1946. Immunity to homologous grafted skin. I. The suppression of cell division in skin grafts transplanted to immunized animals. Brit. J. Exper. Path., **27**: 9–14.

Owen, R. D. 1958. Immunogenetics. Proc. X. Int. Cong. Genetics, **1**: 364–374.

——. 1959. Genetic aspects of tissue transplantation and tolerance. J. Med. Educ., **34**: 366–383.

Rapaport, F. T., L. Thomas, J. M. Converse, and H. S. Lawrence. 1960. The specificity of skin homograft rejection in man. Ann. N. Y. Acad. Sci., **87**: 217–220.

Twitty, V. C. 1959. Migration and speciation in newts. Science, **130**: 1735–1743.

Volpe, E. P. 1957. Genetic aspects of anuran populations. Amer. Nat., **91**: 355–371.

POLYPLOIDY, PHYLOGENY, AND PHOTOPERIODISM IN OLD WORLD *BUDDLEIA*[1]

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Received August 26, 1960

It is commonly accepted that the existence of a world-wide Cretaceous fossil flora of species whose modern relatives are warm temperate or tropical indicates that the climate of this era was warm and remarkably uniform over the entire world. Zonal changes of flora were slight and marked seasonal changes of temperature seem to have been lacking. Woody plants were dominant but it is probable that herbaceous types had already evolved. In the following Tertiary era the type and distribution of plant life changed radically. Apparently a drastic decrease in temperature occurred and zonal segregation of ecological plant types resulted. At this time herbaceous species rose to dominance.

To explain these conditions, Allard (1948) has postulated that the earth's axis was, during the Cretaceous, perpendicular to the plane of its orbit and that consequently the world day was everywhere approximately 12 hours in duration. Such uniform and constant conditions of illumination would certainly promote the remarkable intermingling of ecologically very different species which seems to have been common in this era. With the beginning of the Tertiary the axis slowly departed from the perpendicular and thus arose the seasonal changes in day length with which we are familiar. At the same time the climate generally became cooler and seasonal temperature variations arose. The changes in day length and temperature had a profound effect on the vegetation. Only in the equatorial zone did an almost uniform 12 hour day now occur. Plants which required a short-day period to induce flowering were confined to or driven to the equatorial zone. Long-day or day-neutral plants which may previously have evolved without selective advantage were now able to spread polewards and flourish in the new conditions, relieved of the competition of their short-day relatives. Short-day herbaceous Angiosperms were able to adapt to the seasonal cycle and survive in higher latitudes as fall-flowering plants.

Whether or not a change in the inclination of the earth's axis is accepted as the cause of the Tertiary change in vegetation it is generally accepted that relatively world-wide warm climate characterized the Cretaceous and was succeeded by a cooler, zonal climate. The regions in which tropical species might thrive became narrower as the Tertiary progressed (Stebbins, 1950, p. 525).

Woody dicotyledonous short-day plants are today predominant in the tropics and short-day plants are regarded generally as tropical in origin (Scharfetter, 1953, p. 523). A single species of extensive north-south distribution may be expected to include races with different photoperiodic requirements. Allard (1948) has pointed out that the common potato, *Solanum tuberosum sensu lat.*, is an example of such a species —extending from the short-day regions of Ecuador and Bolivia to Chile, where long days occur in summer. The long-day races which occupy the higher latitudes are regarded as range extensions derived from the short-day tropical races.

If warm-climate woody species were typical of ancient floras and woody short-day species are predominant in the tropics today, it may be suspected that woody short-day species are generally the primitive members of a genus. This criterion must, of course, not be used alone but must

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TABLE 1. *Duration of daylight at various latitudes*

Latitude	Day length (hours, minutes) at middle of month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
10° N	11 36	11 48	12 03	12 20	12 34	12 42	12 40	12 28	12 12	11 56	11 41	11 33
20° N	11 02	11 27	11 59	12 34	13 03	13 20	13 15	12 51	12 18	11 44	11 13	10 56
30° N	10 24	11 05	11 54	12 50	13 37	14 04	13 55	13 17	12 25	11 32	10 42	10 14
40° N	09 36	10 37	11 50	13 10	14 19	15 00	14 47	13 51	12 34	11 16	10 03	09 22
46° N	09 00	10 17	11 46	13 26	14 52	15 42	15 27	14 15	12 41	11 04	09 34	08 42
20° S	13 12	12 48	12 13	11 40	11 10	10 56	11 02	11 23	11 59	12 30	13 03	13 20

be considered together with other data bearing on phylogeny.

The different flowering behavior of groups of *Buddleia* species has previously been noted (Cotton, 1947), and an attempt to correlate flowering habit with evolutionary level and polyploidy may be profitable. Little attention has hitherto been paid to the possible relationship between polyploidy and the flowering habit of angiosperms.

Buddleia is a cosmopolitan genus of 100–150 species found in both the New and Old World between the latitudes 40° N to 40° S. Within this belt, the genus occurs in three areas: North and South America, south and east Africa, and in south central and southeast Asia. Four small closely related genera are found in South Africa. All species are woody perennials: small trees, stout woody lianas, large shrubs, or small xerophytic shrubs.

MATERIALS AND METHODS

Most of the species which it has been possible to obtain in the living condition are Old World species and these alone are discussed here. All are woody perennial shrubs or small trees native to Afghanistan, India, Tibet, southwest China, and the peninsula and islands of southeast Asia between the latitudes 10° to 40° north. Two species are from similar southern latitudes of Africa. One species of the closely related genus *Nicodemia* is included in the study. The genera are separated only by the nature of the fruit and have the same basic chromosome number.

The plants have been cultivated at Ottawa (45° 30′ N), a region with a longer day than that of their native range. The day lengths on the thirteenth day of each month at the latitudes concerned are shown in table 1 (Smithsonian Meteorological tables, 1951). Plants are grown over winter in greenhouse at temperatures of 60°–70° F and experience has shown the conditions necessary to induce optimum growth and flowering. Records of the outdoor winter survival have been kept. The Ottawa area is frost free only from late May to mid-September and temperatures to –30° F occur regularly in winter. A heavy snow cover is present usually from early December. Only one species is sufficiently hardy that the aerial growth is undamaged; many are root-hardy and some never survive outdoors.

From many years of experience the flowering behavior in cultivation has been classified as shown in table 2. From one to three collections of each species were grown. The sources of the material have been previously reported (Moore, 1960). In cases where a species has not been cultivated at Ottawa, observations made in other areas, as indicated, are included. The period of flowering in the native range, as recorded on specimens in the herbarium of the Plant Research Institute, Ottawa and those cited in the monograph of Old World species by Marquand (1930), is also shown in table 2.

Cotton (1947) classed *Buddleia* species in cultivation as either early-flowering species which flower on old wood or as summer-flowering species which flower on

TABLE 2. *Observations of flowering period and hardiness of* Buddleia *species*

Species	Native range	Latitude	Native flowering period	Flowering period in cultivation	Winter-hardiness
		SHORT-DAY			
crispa Benth.	Afghanistan, NW India	30–35° N	Feb.–May	greenhouse, Nov.–Feb.	not hardy
farreri Balf. f. & Sm.	Kansu, Szechwan	30–35° N	Apr.–May	greenhouse, Apr.–May	not hardy
hastata Prain	S Tibet	30° N	?	greenhouse, Apr.–May	not hardy
sterniana Cotton	?	?	?	greenhouse, Apr.	not hardy
alternifolia Maxim.	Tibet–Kansu	30–35° N	Apr.–July	June–July	fully hardy
asiatica Lour.	India–SE Asia	0–30° N	continuous	greenhouse, Nov.–Mar.	not hardy
paniculata Wall.	Assam	25° N	Feb.	greenhouse, Nov.–Feb.	not hardy
salviifolia Lam.	E Africa	0–30° S	continuous	greenhouse, winter (Va.)	?
Nicodemia madagascariensis Parker	Madagascar	20° S	?	greenhouse, Dec.–Mar.	not hardy
japonica Hemsl.	Japan	33–37° N	Aug.–Oct.	June, Sept.	root-hardy
delavayi Gagne.	Yunnan	25° N	May, Aug.	early summer, autumn (Eng.)	not hardy
		SUMMER-FLOWERING			
lindleyana Fort.	E Asia, 103–122° E	27–33° N	June, Nov.	July–Aug. (Va.)	?
candida Dunn	SE Tibet	28° N	Dec.	early Sept.	not hardy
davidii Franch.	Szechwan, Hupeh	30–35° N	July–Oct.	July to frost	root-hardy
fallowiana Balf. f. & Sm.	Yunnan	25–27° N	July–Aug.	July to frost	root-hardy
albiflora Hemsl.	Hupeh, Shensi	30–35° N	July–Aug.	July to frost	root-hardy
nivea Duthie	Szechwan	27–33° N	July–Aug.	July to frost	root-hardy
nivea var. *yunnanensis* Rehd.	Szechwan	27–33° N	July–Oct.	Aug. to frost	root-hardy
forrestii Diels	Yunnan	22–27° N	Aug.–Dec.	July to frost	not hardy
stenostachya Rehd. & Wils.	Szechwan	30° N	Oct.	July to frost	root-hardy
colvilei Hook. f. & Thoms.	Nepal, Tibet	27° N	July, Aug.	June–July (Edinburgh)	?

growth of the current season.

Species here classed as short-day are the following:

Plants which flower in greenhouse at Ottawa during the period November–March under natural day length or with artificial light sufficient to constitute a day of not over 12 hours. These species generally make little growth during summer at Ottawa; rapid growth is resumed from October onwards and buds are formed on new growth in early winter. In their native range these species flower in a 12–13 hour day, either through winter, where temperatures permit, or in early spring.

Plants which flower outdoors early in spring, the flowers appearing on wood of the previous year before or with the leaves. Buds have been formed in the short days of the previous autumn.

Plants which flower at Ottawa on new growth early in the growing season (June) and often flower again late in the year, but not continuously through summer. Perhaps these are intermediate between short-day and long-day or day-neutral; they are here classed as short-day because during the longest days (15 hours) vegetative growth is slight and flowering is sparse or inhibited.

Species classed as summer-flowering grow rapidly and flower freely on new growth from late July until frost. It cannot be stated that these are long-day plants in the usual physiological sense. It appears that flowering is initiated in response to the long days of June but it is possible that the plants are day-neutral and flower at this time only because it is then that sufficient growth has been made. It has been observed that some of these species will flower in greenhouse under natural day illumination in winter. Growth and flowering are greatly accelerated by artificial light sufficient to constitute a 15 hour day. The summer-flowering species are distinguished from those classed as short-day by the fact that growth and flowering of the latter are inhibited by long days or high light intensity and they can be induced to flower only under a day of about 12 hours.

Observations

In table 2 the native range and flowering period, the flowering behavior in cultivation and the winter-hardiness at Ottawa are given for each species.

Cotton (1947) classed as spring-flowering the four species *tibetica, farreri, sterniana,* and *heliophile*. The latter is considered to be synonymous with the prior species *delavayi* (Keenan, personal communication). The three former species, with *crispa,* constitute a closely related group and the present author also classes these as short-day plants. Cotton considered the flowering behavior of *delavayi* (*heliophila*) as unique among known species. In autumn some branches produce flowers, others, resting buds which open in the following spring. In addition, flowers are borne on new growth in early summer and autumn but not throughout summer. Cotton's observations were made in southern England where the maximum day length is 16½ hours, but he noted that Chinese herbarium specimens showed evidence of a similar habit. This species was lost at Ottawa before its flowering habit could be studied. It appears to be a short-day plant which forms buds in short days of spring and autumn. In this, it is similar to *B. japonica,* a species which it resembles in leaf characters.

The species *asiatica* and *salviifolia* have an extensive north–south distribution and specimens have been collected throughout the year. The day length of the native range does not exceed 13 hours and it is probable that these species will flower continuously in relatively short days and mild temperatures.

Buddleia candida is classed as summer-flowering although it does not flower well at Ottawa during July and August. Greenhouse tests have shown that high light intensity will arrest flowering. This species will continue flowering through winter with no or little artificial light and at Edinburgh, where light intensity and temperature are lower, it flowers throughout summer.

Phylogeny in *Buddleia*

The various inflorescence types in this genus have formed the basis of a system of subgeneric classification. They appear to present a unique series of examples of evolutionary trends and afford criteria by which the phylogenetic level of species may be assessed. The evolutionary trends and species representative of various stages have been previously presented (Moore, 1960) and may be briefly reviewed here.

The simplest basic inflorescence from which the present types can be derived appears to be a dichasium of small flowers borne in the axil of normal opposite leaves. An indefinite number of nodes of the terminal portion of a branch may bear flowers. Evolution has progressed by the following trends combined in almost any manner to produce the inflorescence forms seen today:

An increase in the number of flowers in the cluster from 3 to 7, 15 or more.

Change in the length and texture of the flower pedicels and peduncles. This trend, combined with the foregoing, has produced the compact balls of flowers seen in several species.

Change, usually an increase, in the number of floriferous nodes.

Change, usually a decrease, in the length of internodes of the floriferous branch.

Reduction in size of the leaves subtending the flower clusters progressively toward the apex of the branch to, finally, minute bracts.

Branching of the inflorescence. This may be visualized as a development from the primitive simple flowering stem whereby, in the place of dichasia, arose branches which, in turn, bear flower clusters.

On the basis of this theory of inflorescence evolution the species are evaluated as "primitive," "intermediate," or "advanced." Examples of the meaning of these terms are the following.

Primitive.—*B. crispa*: inflorescence consists of a few nodes, cyme consists of 7–15 flowers with no special organization, leaves subtending cymes are only partially reduced. *B. alternifolia*: inflorescence is branched but branches are reduced to spurs, cluster contains a small number of flowers, the subtending leaves are only slightly reduced.

Intermediate.—*B. paniculata*: inflorescence branched, cyme contains many flowers organized in globular form, subtending leaves reduced to bracts.

Advanced. — The spicate inflorescence seen in many species is considered to be the most advanced. The number of floriferous nodes is great and the leaves are reduced to minute bracts. The flowers may be borne in more or less sessile dichasia (*B. asiatica*) or in pedunculate cymes (*B. davidii*). Further specialization is seen in the curved corolla of *B. japonica*, *B. lindleyana*.

Cytology

The chromosome number of these species has been reported by Moore (1960). The basic haploid number of *Buddleia* and of *Nicodemia* is 19 and species with the 2x, 4x, 6x, 12x, and 16x levels of ploidy are included in the foregoing. To summarize the above data, in table 3 are listed the species, with their chromosome number, photoperiodic requirement and an evaluation of their phylogenetic level.

Discussion

The interrelationships of polyploidy, phylogenetic level, photoperiodism, and cold-hardiness of the various species with the geographical distribution and general evolution of the genus remain to be considered.

The most obvious correlation appears in the fact that almost all the diploid species are short-day plants and that most known polyploids flower in long days.

Polyploid species are generally recognized as more recent in origin than diploids, for it is obvious that the former have been derived from diploid stock. The positive correlation in the majority of species between the polyploid condition and an inflorescence type evaluated as "advanced" is therefore significant. Differ-

TABLE 3. *Comparison of polyploidy, photoperiodism and phylogenetic level of some species of* Buddleia

Species	Degree of ploidy	Photoperiodism	Phylogenetic level
		Diploid Species	
alternifolia	2x	short-day	intermediate
asiatica	2x	short-day	advanced
crispa	2x	short-day	primitive
farreri	2x	short-day	primitive
hastata	2x	short-day	primitive
japonica	2x	short-day ?	advanced
lindleyana	2x	summer-flowering	advanced
paniculata	2x	short-day	intermediate
sterniana	2x	short-day	primitive
salviifolia	2x	short-day	primitive
Nicodemia madagascariensis	2x	short-day	intermediate
		Polyploid Species	
albiflora	6x	summer-flowering	advanced
candida	4x	summer-flowering	advanced
colvilei	16x	summer-flowering	advanced
davidii	4x	summer-flowering	advanced
delavayi	6x	short-day	intermediate
fallowiana	4x	summer-flowering	advanced
forrestii	6x	summer-flowering	advanced
nivea	6x	summer-flowering	advanced
nivea var. *yunnanensis*	12x	summer-flowering	advanced
stenostachya	6x	summer-flowering	advanced

ences in level of evolutionary development are to be expected among the diploids, and it is not surprising that some diploid stock has reached an advanced morphological level. It is conceivable that polyploids may have evolved from diploids of either primitive or advanced morphological types and that some polyploids possess an unbranched spicate inflorescence and appear to proceed on from the level of advanced diploids.

It is concluded that all diploid and one polyploid species of low or intermediate phylogenetic level are short-day, some morphologically advanced diploids are day-neutral and all known polyploids of advanced morphology flower in long days. Trends to the summer-flowering habit are seen in some diploids with the advanced spicate inflorescence. It is not surprising that an offshoot of short-day stock (*B. delavayi*) has become polyploid. From study of the morphology of the species and of numerous artificial interspecific hybrids the author (unpublished) believes that several diploid stocks have entered into the formation of the Asiatic polyploids.

The question arises whether the change from short-day to summer-flowering habit is to be attributed to polyploidy *per se* or to gene changes accompanying evolutionary advance. Little attention has been paid to this question, though there exist some studies of flowering behavior in relation to polyploidy. Sax (1936) has pointed out that most Old World species of *Spiraea* are early-flowering diploids and that all American species are late-flowering. All of the latter species examined cytologically are tetraploids. He concluded that polyploidy alone was not responsible for the delay in flowering time, for the tetraploid variety of *S. chamaedryfolia* is little later than the diploid parent. The sole hexaploid species flowers relatively early. Most oriental species of *Malus* are diploid (Sax, *l.c.*), whereas most American species are tetraploid. The former flower earlier than the latter and, in the Arnold Arboretum, the flowering periods do not overlap. Löve

and Löve (1949) state that a diploid species of *Acetosella* is a short-day plant and that a tetraploid and a hexaploid species are long-day.

The flowering behavior of diploids and their artificial tetraploid derivatives have been compared by various authors and, on the whole, no significant difference was found. Müntzing (1936) reviewed the work of several authors with *Crepis capillaris*, cultivated tomato, *Cleome* spp. and *Canna* spp. comparing growth rate and time of flower initiation in diploids and in their artificially produced tetraploids. He compared also the physiology of natural and artificial polyploids. He concluded that in almost every case the growth rate of the artificial tetraploid was slower than that of the diploid and that consequently flower initiation was retarded, by as much as 45 days in the case of the tomato. Natural and artificial polyploids were found to be morphologically and physiologically similar and the natural polyploids had a longer vegetative period and flowered later than the diploid. Kostoff and Kendall (1931) reported that a tetraploid of *Petunia violacea* grew faster and flowered 8 days earlier than the diploid. More recent work has confirmed the general conclusion that the flowering behavior of artificially produced tetraploids does not differ significantly from that of their diploid progenitors: Lona (1947) with *Xanthium italicum*, Lang (1947) for *Hyoscyamus niger*, *Antirrhinum maius* and *Xanthium strumarium*, Vaarama (1947) with *Ribes nigrum* and Müntzing (1951) with *Secale cereale*. The slight difference in date of initiation of flowering between the diploids and tetraploids cannot be regarded as a change in the photoperiodism of the species, but more likely as due to a different rate of growth.

From the foregoing studies and from the observations on *Buddleia* that the summer-flowering habit is not confined to the polyploids and that most short-day diploids are classed in the lower phylogenetic levels, it appears more reasonable to conclude that the change from short-day to the summer-flowering habit has accompanied evolutionary advance and is not the result of polyploidy *per se*.

The photoperiodic response of a species has great bearing on its geographical distribution, past, present, and potential. Migration into the higher latitudes will be open only to species which have developed day-neutral or long-day races or have been able to adapt the short-day habit, by virtue of other advantageous characters that they may possess, to conditions in the long-day area. In the genus *Buddleia* the most northerly and most cold-resistant Asiatic species are diploids: *B. alternifolia*, *B. japonica*, and *B. lindleyana*. These species, although chiefly short-day, have been able to adapt their flowering habits to northern conditions by utilizing their greater hardiness. *Buddleia alternifolia* initiates flower formation in the short days of autumn, the flowers opening in the following spring. Were the species not cold-hardy the stems and buds would be unable to survive the winter. *Buddleia japonica*, almost as hardy as *B. alternifolia*, is able to flower early in the year because it begins growth very early and presumably forms buds in June before the day length has become prohibitively great. Polyploid long-day species are better equipped, so far as photoperiodism is concerned, to advance into northern latitudes than are the diploids. The lack of very hardy races apparently has restricted their spread to date. It is possible that the polyploids would be able to flower in a more southerly short-day area, aided by higher temperature, but they would enjoy no advantage and probably some disadvantage as a result of their flowering habit. The present distribution of the polyploid species is not extensive; probably the group is still youthful and has spread little beyond the point of origin.

Over the past 30 years a great deal of interest has been shown in the question of polyploidy as related to plant distribution. Numerous authors have examined the relative frequency of diploids and polyploids

in the floras of certain areas and have noted correlations between the frequency of polyploids and such factors as latitude, severity of climate, and age of the flora. Although, in the present case, the different photoperiodic response characteristic of the majority of the diploids and of the polyploids has not yet caused a distinct latitudinal segregation, it is apparent that flowering habit correlated with phylogenetic level and polyploidy could be an important factor in influencing the relative frequency of diploid and polyploid species in a flora extending over a considerable latitude. The short-day habit of diploids may constitute a barrier which, although not insurmountable, may be of considerable significance in confining these species to the lower latitudes. The long day of higher latitudes might be expected to encourage the migration of long-day polyploids to zones where their physiological adaptation will be most advantageous.

The relationship of photoperiod to the spread of *Buddleia* in the past is significant. Diploid species occur in the New World, east Africa, and southeast Asia, all of the areas in which the genus is found today, and it is obvious that the genus has spread in the diploid condition. If the photoperiodic nature of Old World species is characteristic of species of all areas, it follows that the primitive diploid species were short-day plants. Dissemination could then have taken place only through regions where the summer day length was short, 12–13 hours. With the present inclination of the earth's axis, this condition is found only between 15 degrees north and south of the equator. Migration by a circumpolar route is impossible, even if temperature and other conditions were suitable, and it must be concluded that the genus spread across the Atlantic in the tropical zone. Whether land bridges existed or whether the continents were in contact in this area in sufficiently recent time is a much debated question. However, dissemination by air-borne seed seems quite possible in the case of *Buddleia*. The seeds are minute and bear narrow wings and doubtless would be transported over a great distance if carried upwards to a considerable height.

In this connection, the conclusions of Axelrod (1959) with regard to the region of origin of the angiosperms is of interest. From a study of the frequency of angiosperm fossils at different geological ages and at different latitudes he concluded that angiosperms arose in pre-Cretaceous time in the uplands of an equatorial belt between 45° N and 45° S latitude. Angiosperms entered the lowlands of this zone in early Cretaceous and reached higher latitudes by the end of the era.

Only if a Cretaceous world-wide day of 12 hours is accepted would migration through the higher latitudes be possible. In the absence of a fossil record the geologic time of origin of the genus cannot be established and it cannot be denied that the most primitive species reached the present land masses of America, Africa, and Asia at such an early date. However, the advanced features of floral morphology of *Buddleia* and the fact that the flowers are adapted to pollination by the most advanced and specialized insects (Hymenoptera, Lepidoptera) suggest that the genus originated at a later date, perhaps early Tertiary, at a time when climatic segregation of floras was beginning and when, presumably, seasonal variation in day length occurred. Under such conditions, the conclusion seems inescapable that *Buddleia* has spread across the world through the equatorial zone.

Summary

The flowering habit of 21 Old World species of *Buddleia* in cultivation and in their native habitat is described, and they are classed as either short-day or as summer-flowering plants. The chromosome number, cold-hardiness and phylogenetic level, as evaluated on the basis of inflorescence evolution, are stated for each species. Nine of the 10 polyploid species flower in long days, while 10 of the 11 diploid spe-

cies flower in short days; one diploid of advanced morphology flowers in summer; one polyploid of intermediate evolutionary rank is a short-day species. Diploid species are classed as primitive, intermediate, or advanced with regard to phylogenetic level; most polyploids are classed as advanced. It is concluded that the short-day photoperiodic habit is correlated with a less advanced phylogenetic level; change to the summer-flowering habit accompanies evolutionary advance. Polyploidy does not change the photoperiodic habit. Photoperiodic response has not yet led to a latitudinal segregation of diploids and polyploids. The summer-flowering polyploid species have not spread into the higher latitudes presumably because they lack cold hardiness. Several diploid species are the most hardy known and these are the most northerly of the genus. It is postulated that the primitive stock consisted of short-day diploid species, and that it has spread through the equatorial zone.

Acknowledgment

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Literature Cited

Allard, H. A. 1948. Length of day in the climates of past geological ages and its possible effects upon changes in plant life. *In* A. E. Murneek and R. O. Whyte, Vernalization and photoperiodism. Lotsya, Vol. 1. Chronica Botanica Co., Waltham, Mass.

Axelrod, D. I. 1959. Poleward migration of early Angiosperm flora. Science, **130**: 203–7.

Cotton, A. D. 1947. The spring-flowering *Buddleias*. J. Roy. Hort. Soc., **72**: 427–37.

Kostoff, D., and J. Kendall. 1931. Studies on certain *Petunia* aberrants. J. Genet., **24**: 165–178.

Lang, A. 1947. Beiträge zur Genetik des Photoperiodismus II. Photoperiodismus und Autopolyploidie. Zeitschr. Naturforsch., **2b**: 36–44.

Lona, F. 1947. Esigenze fotoperiodiche dei poliploidi e loro significato ecologico e fitogeografico. Nota preventiva. Nuovo G. Bot. Ital., **54**: 793–96.

Löve, A., and D. Löve. 1949. The geobotanical significance of polyploidy. Portugaliae Acta Biologica, Ser. A., R. B. Goldschmidt Vol., pp. 273–352.

Marquand, C. V. B. 1930. Revision of the Old World species of *Buddleia*. Kew Bull. Misc. Inf., No. **5**: 177–208.

Moore, R. J. 1960. Cytotaxonomic notes on *Buddleia*. Amer. J. Bot., **47**: 511–517.

Müntzing, A. 1936. The evolutionary significance of autopolyploidy. Hereditas, **21**: 263–378.

——. 1951. Cytogenetic properties and practical value of tetraploid rye. Hereditas, **37**: 17–84.

Sax, K. 1936. Polyploidy and distribution in *Spiraea*. J. Arn. Arb., **17**: 352–56.

Scharfetter, R. 1953. Biographien von Pflanzensippen. Springer Verlag, Vienna.

Stebbins, G. L. 1950. Variation and evolution in plants. Columbia Univ. Press, New York.

Vaarama, A. 1947. Morphological and cytological studies on colchicine-induced tetraploid *Ribes nigrum*. Suomen Maataloustieteellisen Seuran Julkaisusta, **67**: 55–93.

A SALAMANDER COLOR VARIANT ASSOCIATED WITH GLACIAL BOUNDARIES[1]

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The range of the red-backed salamander, *Plethodon cinereus*[2] (except for outlying areas), is roughly indicated by a triangle bounded by the Atlantic Ocean on one side, a line extending from the mouth of the St. Lawrence Seaway past Duluth, Minnesota, to the Mississippi River, and another line extending from there southeast to the North and South Carolina border at the ocean (see Conant, 1958, map 179 for details). Viewed from above, this small slender salamander is usually black with a red dorsal band on the head, back, and tail.

The color variant in question appears grossly to be an all-red *cinereus*, with the usual black pigmentation restricted to the eyes. This is the "erythristic phase," a name coined by Barbour (1914), and used by others such as Mathews (1952). The agreement between the latter's detailed descriptions and those of others, particularly Reed (1908), indicates that this color variant is a definite entity. It is not a temporary condition, as animals have been observed in captivity for longer than a year (C. F. Reed, 1955). It is also clear that the "erythristic" appearance is more apparent than real, since the greatest change is a decrease in black pigment rather than an increase in red. Examination of many live *cinereus* (Thurow, 1955, 1956, and subsequently) confirms that the red pigment is much more extensive than the dorsal stripe in most striped examples, but that the red is largely masked by black pigment outside of the stripe. The descriptions of the erythristic form are not as clear concerning changes in the white or "guanophore" pigment. C. F. Reed was kind enough to show me a live example of this form and color photographs of additional individuals. There was no marked reduction of the white pigment of these animals, contrary to the impression gained from his own description (1955), and that of H. D. Reed (1908). The word "immaculate" used by both these authors to describe the ventral surface, applies only to the lack of black pigment, not white. The ventral surfaces of the *living* erythristic form should be examined with a binocular dissecting microscope to determine whether the "pink" color described by the same two authors is actually due to red pigment cells in the skin of the belly. These cells do not characteristically occur here in *P. cinereus*, and the pink hue could be due to light filtered through the dorsolateral red pigment and the translucent tissues of the living body.

Mathews (1952) reviewed the literature on the erythristic form. C. F. Reed (1955) described additional specimens from Litchfield County, Connecticut. An additional locality was reported by Bleakney and Cook (1957).

DATA

The locality records shown in figure 1 are listed below. Mathews (1952) is the most convenient summarizing reference for further details. The records listed below that are not given by Mathews or C. F. Reed (1955) are indicated by asterisks. In figure 1 the records are located by dots within heavy circles, which are sometimes incomplete so as not to obscure map names. There is no dot within the Worcester County circle because of the inexact locality. Similarly, an ellipse rather than a dot indicates the locality near Buffalo, which

[1] This paper is a part of a doctoral dissertation (Thurow, 1955), with minor changes.

[2] Since this paper was submitted for publication the name *Plethodon cinereus* (Green) has been changed to *P. erythronotus* (Raf.) by C. F. Reed (Herpetologica, **16**: 207–213, 1960).

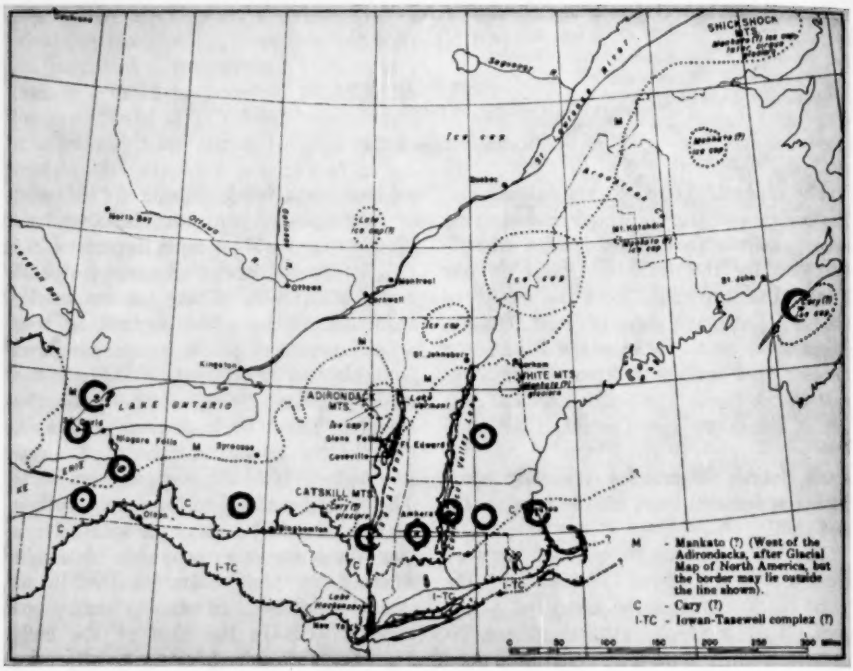
SHICKSHOCK MTS.
LAKE ONTARIO
ADIRONDACK MTS.
CATSKILL MTS.
WHITE MTS.
M - Mankato (?) (West of the Adirondacks, after Glacial Map of North America, but the border may lie outside the line shown).
C - Cary (?)
I-TC - Iowan-Tazewell complex (?)

FIG. 1. Locality records for the erythristic form of *P. cinereus* plotted on a map showing Wisconsin glacial borders in eastern North America (adapted from Flint, 1953).

was deduced partly from the altitude. A question mark indicates the status of the Toronto record. To facilitate identification the individual records are listed by states and roughly from west to east, or from left to right, as they appear in figure 1.

CANADA

ONTARIO: About 7 mi E of Hamilton, just above (S of) the Niagaran Escarpment at Albion Falls*—3, "a very rare phase" (Brown, 1928, pp. 125–7); within 15 mi of Toronto*—1 (Piersol, 1909, p. 469. This is a questionable record, since the remark was merely made that 1 out of about 250 had much more than the usual amount of red.)

NOVA SCOTIA: *Annapolis Co.*, On the shore of Sandy Lake, on North Mountain, N of Paradise*—2 (Bleakney and Cook, 1957). Over 250 other *P. cinereus* from Nova Scotia were not erythristic.

UNITED STATES

NEW YORK: *Chautauqua Co.*, Chautauqua—1 (H. D. Reed and Wright, 1909, p. 403). *Erie Co.*, Buffalo (2 localities in hills to SE)—2 (H. D. Reed, 1908, p. 460). *Tompkins Co.*, Besemers (SE of Ithaca)—1 (ibid.). *Ulster Co.*, New Salem—1 (Bishop, 1941, p. 200).

CONNECTICUT: *Litchfield Co.*, near North Colebrook (2 mi N of Colebrook)—16 erythristic phase individuals together with 54 striped (Mathews, 1952, p. 277); Highland Lake—15, with 85 striped and 2 leadbacked (C. F. Reed, 1955); Burr Pond State Park, near Burrsville—5, with 1 striped taken in the same general region (ibid.).

MASSACHUSETTS: *Hampshire Co.*, Amherst—1, with 7 striped (Bartlett, 1952, p. 100); *Worcester Co.* (probably near Worcester)* — 2, "in company with several normal ones, in widely separated spots" (Cochran, 1911, p. 336); *Norfolk Co.*, Cohasset—1 (Barbour, 1914, pp. 3–4).

NEW HAMPSHIRE: *Merrimack Co.*, Andover—1, with 7 striped (Burt, 1945, p. 204).

When these localities are plotted on a map they form a rough line north of the generalized boundary of the Wisconsin glaciation, which passes through Pennsylvania and New Jersey. This distribution can be correlated with more recent Wisconsin glaciations that terminated in the general area. Thanks to Dr. R. F. Flint I am able to reproduce his map (Flint, 1953) showing these glacial patterns, with the erythristic phase localities superimposed on them (fig. 1). The Litchfield County records are all indicated by one dot and a surrounding heavy circle with two protuberances on it, so only twelve different localities are shown. Eight of these lie close to the border of the Cary glaciation, seven just north of it and one just to the south, or perhaps just to the north (see Hyyppä, 1955, fig. 1). The New Hampshire record lies about midway between the Cary and Mankato boundaries, the Buffalo record lies about on the Mankato margin, and the Hamilton and questionable Toronto records lie north of and inside this boundary.

There is evidence that the localities shown in fig. 1 actually represent the distribution of the erythristic form. It is a very conspicuous phase, and *P. cinereus* is a common and abundant species described as long ago as 1818. It is well represented in museum collections. Collecting in northeastern United States has been intense because of the high human population density there. Burger (1935) collected heavily just south of the Tazewell (and Wisconsin) glacial border in eastern Pennsylvania and adjoining New Jersey, listing 1,032 specimens, and yet he found no definite erythristic forms although he described other variations. Bishop and H. D. Reed, who both reported all the erythristic records from New York, collected extensively in this state. Their work also indicates that this color variant is quite rare, and essentially absent in most of New York. In my thesis, and subsequently, I reviewed the literature and museum collections of *P. cinereus* over its entire range and found no other records. Mathews reported most of the erythristic records in 1952, and I described the glacial pattern in 1954 (paper read at Florida A.I.B.S. meetings) and in 1955 (Thurow, 1955). Yet by 1960 the only additional records (C. F. Reed, 1955, and Bleakney and Cook, 1957) confirm rather than change the distribution pattern.

DISCUSSION

The nature of the erythristic form and its distribution suggest that it is the result of genetic rather than environmental factors. The simplest interpretation is that it is the phenotype resulting from the action of a mutant allele that quantitatively inhibits the development of melanin (or possibly melanophores). To use *Drosophila* terminology, the phenotype is clearly not the normal or wild type, and such a marked melanin reduction is not characteristic for any species of *Plethodon*. In species for which breeding data are available (e.g. *Drosophila*, mice, guinea pigs, and mink) such non-wild type body pigmentations are due to alleles that are more often recessive than dominant in action.

Some intermediates have been described (H. D. Reed, 1908), and it is possible that there is only partial dominance and these represent heterozygotes, or that more than one locus is involved. This may be questioned, however, without breeding data. H. D. Reed (ibid.) looked at "several hundred" animals, mostly from the Cayuga Lake basin in New York, and picked out a series of 15 that "show a fairly complete transition" from the all black phase through the common striped form to the erythristic specimens that he found near Besemers (in the Cayuga Lake basin) and Buffalo. Such

a series, shown in his figures 1–7, appears to be an artificial arrangement. His only erythristic "intermediate" (fig. 5) can be interpreted as within the range of variation of the striped phase. "Intermediates" would seem to belong better between his figures 5 and 6. In approximately 6,000 *cinereus* that I examined the only erythristic phase example was one from Litchfield County, Conn., and only about a half dozen could be interpreted as showing a marked leaning in this direction. In this material there is no continuous gradation between erythristic and striped forms, as interpreted by Reed. The variation in the development of black skin pigment described by Mathews (1952) and shown by figures 6 and 7 of H. D. Reed (1908), may be explained by the phenomenon of expressivity (see Winchester, 1958, pp. 290–293). Some individuals from Hellertown, Penn., mentioned by Burger (1935, p. 582), may represent a different genetic factor with a similar but less marked action.

Records of juveniles of the erythristic form should be checked carefully, since many salamanders undergo marked changes in pigmentation for a short period after transformation (or hatching). This is supported for *P. cinereus* by the observations of Smith (1882), who says that newborn individuals are usually like the adults, but are often bright red, spotted with black. This slight delay in the normal development of the black pigment may give some indication of the mechanism involved in the arrested development of black pigment in the erythristic phase. If the red pigment is a precursor to the black, a change in a single enzyme could be involved (see Hertzler, 1951).

The erythristic variant must have a frequency of less than 1% in the general *cinereus* population, even in the area shown in fig. 1. The frequency would almost certainly be less than 5% within all the local breeding populations from which the erythristic form has been recorded, with the exception of the Litchfield County localities. These statements are based not only on the sampling data listed with the locality records of this conspicuous phase, but on the mass of negative collecting partially described below the locality records. In addition, table 1 of Thurow (1955) indicates that more than 1,806 specimens were examined from the part of the United States shown here in fig. 1. Of these, 943 were from New York, and 273 of these were a series taken from Allegheny State Park, Chautauqua Co., not far from the Chautauqua County erythristic record. New England, exclusive of Maine, was the source of 413 of these specimens, and 402 or more came from the part of Pennsylvania shown in fig. 1. Still more negative collecting data supporting the rarity of the erythristic form is in the form of literature records in tables 1 and 2 of Thurow (1955). Of these, the black phase records, at least, are unquestionably not the erythristic form.

The observed distribution of the frequency of the erythristic phase may be explained on the basis of genetic drift, in a situation somewhat similar to that described by Elton for the arctic fox (reviewed by Emerson, 1949). The work of Elton and others (see discussion in Dobzhansky, 1947, pp. 172–175) shows that areal restrictions such as islands and peninsulas favor random genetic changes. This correlates with the tendency for allele frequencies to approach either 0 or 100% at random in small inbreeding populations (Wright, 1932, and elsewhere). This more random gene fixation would give relatively high frequencies of the allele in some populations, and the result would tend to be a rise in the net frequency of a rare recessive phenotype, regardless of its fate in any particular local population. Although the rare phenotype would be lost much more often than it would be fixed, the magnitudes of increase in phenotype frequency are much greater here than those of decrease. Negative collecting data cited above, and the collecting of Mathews (1952) and C. F. Reed (1955), suggest strongly that the higher phase frequencies reported here are indeed "spotty," rather than a continuous uniform band.

Elton's ideas of random extinction and random re-establishment, and Sewall Wright's work on the ascendancy of genetic drift over selection in small populations, are combined in Wright's ideal conditions for the fixation of random genetic changes—"frequent extinction of the populations of small isolated localities with restoration from the progeny of occasional stray migrants from other localities" (Emerson, 1949, p. 603). This situation well fits the usually forest-restricted *P. cinereus* (Shelford, 1937; Grobman, 1944; my own field observations, and those of many others). The species probably occupied small islands and peninsulas of suitable forest advancing over the recently glaciated territory, and was kept back and sometimes killed back by the oscillating front of the glacier. Such a situation would favor the chance establishment of rare mutations in such advance forest "islands," and would facilitate their spread along these "islands" parallel to the glacial front. These "islands" are not the physical islands of coniferous forest at the extreme northern forest boundaries, but rather the suitable areas of moist forest, well behind the pioneer tree types. The distribution of the erythristic form makes it quite probable that it was established in the species population after the Cary ice retreat, and perhaps during the Mankato maximum. That is, that the records north of the Cary boundary indicate a higher gene frequency established in the forest advancing over the area vacated by the Cary glacier.

A further refinement of the interpretation of erythristic phase distribution is possible along these same lines. Litchfield County, Conn., the one place where the frequency of the variant is markedly higher, lies within an area that was isolated to an even greater degree. Mathews took 70 specimens from North Colebrook, 22.8% being erythristic. C. F. Reed collected 102 from Highland Lake, 14.7% of which were erythristic. Except for two of Reed's specimens from Lake Waramaug near New Preston, all the collecting of these two authors was in a small area centering around Winstead. Their total collection in this area is 194 specimens, 18.6% of which are the erythristic phase. Fig. 1 shows that the Litchfield County locality (indicated by the circle with the vertical protuberances) was isolated between two long lakes formed in the Hudson and Connecticut valleys as the Cary ice retreated. It is possible that the original establishment of the erythristic form took place between these two lakes in the Cary–Mankato interval before the western lake was drained (see Flint, 1953, p. 903), or that it occurred after this time, when the area was still ecologically isolated for plethodontid salamanders.

Regardless of the exact time and place of the establishment of the erythristic phase, the New Hampshire record and the Buffalo and more western records definitely indicate a spread or radiation from the general Connecticut area that occurred *after* the Mankato maximum to a large degree, because these records closely approach and even overlap the Mankato border. The fact that these more western records lie farther north with respect to glacial boundaries may indicate that the erythristic form spread gradually westward, and was established in territory recently abandoned by the Mankato glacier in the same way as it was established after the Cary retreat.

Fenneman (1958, fig. 91) shows the position of the ice and glacial lakes in New York after the waning of the Mankato ice was well begun. Recent opinion (see Braun et al., 1951) is that, although coniferous forests may have occurred through much of New York at this time, partly deciduous forests presently more suitable for *P. cinereus* had not yet advanced beyond the southeastern corner of the state. However, under the cooler and moister climate of that time *cinereus* may have been able to colonize these pioneer coniferous forests to some degree, as it does presently in the northern extremes of its range.

The Nova Scotia locality suggests that the color variant may also have spread

eastward along the Cary boundary. Populations between these areas have been wiped out by a rising sea level, or records may still be found along the coast of Maine and New Brunswick, as there has been less collecting in these areas.

There is no corresponding southward spread of the erythristic form from the main line of concentration. This could be explained by assuming that this form has a negative selective value compared to the striped form or the black form, and that the former was not able to replace these types in the larger, more continuous populations to the south. There are no experimental data, but field experience with *P. cinereus* indicates that the erythristic form would be more conspicuous to diurnal predators at least, whether they have color vision or not. After its sheltering log, or rock, etc. is turned the outline of this nearly all-red form contrasts more strongly with the background, which is usually darker. The rareness of this variant also suggests either a low selection value, a low mutation rate, or both. It may be that gradual submergence of this form was initiated once fairly continuous salamander populations were re-established. Such a trend would tend to be halted by the human modifications of the forests that occurred about 150 years ago, once again producing many small isolated colonies.

If the series of events postulated here is correct, and if the radiocarbon dates of these glaciations are accurate (see Flint, 1953; Flint and Rubin, 1955; and other publications), then the erythristic form became established not earlier than about 13,000 years ago and started to spread actively no more than about 10,000 years ago. Active spread probably ceased more than 5,000 years ago, and the variant may have been partly submerged since, although the human deforestation of historical times would tend to preserve it.

The radiocarbon dates and the above estimates may already need revision as this is published. The point is that it appears feasible to make reasonably accurate determinations of the time of the glacial events in question. The history of the assumed prehistoric genetic changes in this species can be dated with better accuracy than in most such cases. It is also possible to confirm and further analyze the genetic nature of this color variant with field and laboratory methods now in existence.

Summary

The erythristic form of *Plethodon cinereus* is further described. The nature and distribution of this color variant are such that it is assumed to be due to genetic rather than environmental modification, possibly a single recessive allele inhibiting melanin formation.

Locality records for this form are plotted upon a map showing glacial features in northeastern United States and adjacent Canada. Most of the records are in a line a little inside the limit of the Cary glaciation. The hypothesis is presented that the frequency of the rare allele (or allele combination) assumed to be responsible for the variant was increased in certain areas along the oscillating glacial margin by random extinction and recolonization among small semi-isolated populations. Random fixation of a rare allele like this (probably recessive and with negative selective value) in such small colonies would result in some large increases in frequency of the erythristic variant, and would tend to raise the total net frequency of this form. This mechanism of genetic change appears to be consistent with the ecology of the situation, and with the present distribution of the erythristic form. A further application of this principle suggests that the center of origin for this color variant was in Litchfield County, Connecticut. Evidence indicates that the form originated after the Cary glaciation, and spread to approximately its present distribution during and after the Mankato glaciation. Since the glacial events in question have been radiocarbon dated, it is possible to estimate the sequence of events in terms of years. Further analysis of this interesting case awaits

a more detailed local glacial chronology and an analysis of the genetics of the erythristic color variant.

Acknowledgments

I am indebted to the Zoology Department of Indiana University where this work was carried out, both for facilities and for research funds to pay for photography for figure 1, and to Dr. F. N. Young, the chairman of my doctoral committee. Dr. R. F. Flint gave permission to reproduce the glacial map in his 1953 paper. Time to publish this part of my thesis was provided by Dr. M. H. Knisely, head of the Anatomy Department of the Medical College of South Carolina, through U. S. Public Health Service Grant H.T.S. 5340. Dr. Elsie Taber and Dr. Sewall Wright read drafts of the manuscript and made suggestions.

Literature Cited

Barbour, T. 1914. An unusual red salamander. Copeia, **10**: 3–4.

Bartlett, L. M. 1952. An erythristic *Plethodon c. cinereus*. Herpetologica, **8**: 100.

Bishop, S. C. 1941. The salamanders of New York. Bull. N. Y. St. Mus., **324**: 1–365.

Bleakney, S., and F. Cook. 1957. Two erythristic *Plethodon cinereus* from Nova Scotia. Copeia, **1957**: 143.

Braun, E. L. (chairman), and H. N. Raup, C. S. Denny, J. E. Potzger, J. N. Wolfe, and E. S. Thomas. 1951. The glacial border—climatic, soil, and biotic features. A symposium of the Ecological Society of America, held at Columbus, Ohio, in 1950. Ohio J. Sci., **51**: 105–146.

Brown, J. R. 1928. The herpetology of Hamilton, Ontario, and district. Canad. Field Nat., **42**: 125–127.

Burger, J. W. 1935. *Plethodon cinereus* (Green) in eastern Pennsylvania and New Jersey. Amer. Nat., **69**: 578–586.

Burt, C. E. 1945. A mutant red phase wood salamander (*Plethodon cinereus*) from New Hampshire. T. Kans. Acad. Sci., **48**: 204.

Cochran, M. E. 1911. The biology of the red-backed salamander (*Plethodon cinereus erythronotus* Green). Biol. Bull., **20**: 332–349.

Conant, R. 1958. A field guide to reptiles and amphibians. Houghton Mifflin Co., Boston, 366 pp.

Dobzhansky, T. 1947. Genetics and the origin of species (2nd ed.). Columbia Univ. Press, New York, 446 pp.

Emerson, A. E. 1949. Ecology and Genetic Variation. (Pp. 599–605 in *Principles of Animal Ecology* by Allee et al. W. B. Saunders Co., Philadelphia, 837 pp.)

Fennemann, N. M. 1938. Physiography of eastern United States. McGraw-Hill Co., New York, 714 pp.

Flint, R. F. 1953. Probable Wisconsin substages and late-Wisconsin events in northeastern United States and southeastern Canada. Bull. Geol. Soc. Amer., **64**: 897–920.

—— and M. Rubin. 1955. Radiocarbon dates of pre-Mankato events in eastern and central North America. Science, **121**: 649–658.

Grobman, A. B. 1944. The distribution of the salamanders of the genus *Plethodon* in the eastern United States and Canada. Ann. N. Y. Acad. Sci., **45**: 261–316.

Hyyppä, E. 1955. On the Pleistocene geology of southeastern New England. Bull. Comm. Geol. Finl., **167**: 155–225.

Hertzler, E. C. 1951. The red and black pigment of the salamander, *Plethodon cinereus* (Green). Ph.D. thesis Univ. Mich.

Mathews, E. 1952. Erythrism in the salamander *Plethodon cinereus*. Copeia, **1952**: 277–278.

Piersol, W. H. 1909. The habits and larval state of *Plethodon cinereus erythronotus*. T. Roy. Can. Inst., **8**: 469–493.

Reed, C. F. 1955. Notes on salamanders from western Connecticut, with especial reference to *Plethodon cinereus*. Copeia, **1955**: 253–254.

Reed, H. D. 1908. A note on the coloration of *Plethodon cinereus*. Amer. Nat., **42**: 460–464.

—— and A. H. Wright. 1909. The vertebrates of the Cayuga Lake basin, New York. Proc. Amer. Phil. Soc., **48**: 370–459.

Shelford, V. E. 1937. Animal communities in temperate America. Univ. Chicago Press, Chicago, 368 pp.

Smith, W. H. 1882. Report on the amphibians and reptiles of Ohio. Rep. Geol. Surv. Ohio, **4**: 629–734.

Thurow, G. R. 1955. Taxonomic and ecological studies on the zig-zag salamander (*Plethodon dorsalis*) and the red-backed salamander (*Plethodon cinereus*). Ph.D. thesis Indiana Univ.

——. 1956. Comparisons of two species of salamanders, *Plethodon cinereus* and *Plethodon dorsalis*. Herpetologica, **12**: 177–182.

Winchester, A. M. 1958. Genetics. Houghton Mifflin Co., Boston, 414 pp.

Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution. Proc. Sixth Internat. Congr. Genetics, **1**: 356–366.

CONVERGENCE AND PARALLELISM IN CAVE ENTOMOBRYINAE

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Convergence and parallelism are of great interest to the student of evolution but detailed expositions of these processes involving extant animals are rare. The Collembola of caves furnish an excellent and unexploited field for studies of this and other evolutionary problems, since these forms are common in caves, ubiquitous, and have gone through extensive evolution within caves. The work that follows[1] is a first attempt to examine the evolution of one group of cave Collembola, the subfamily Entomobryinae. Using this term in its narrow sense, there are two genera found commonly in caves: *Sinella* and *Pseudosinella*. In both cases special genera have been created for some of the cave species, but these genera (*Parasinella*, *Coecobrya*, *Troglosinella*, and *Sirodes*) are indistinguishable from the two genera mentioned above and are not considered as separate here. In addition to the members of these two genera, a few species of the genus *Entomobrya* are apparent troglobites, and a scattering of troglophile or trogloxene species are found in this genus and in the genera *Entomobryoides*, *Calx*, *Drepanura*, *Lepidocyrtinus*, and *Lepidocyrtus*.

Both *Sinella* and *Pseudosinella* are patently polyphyletic groups, and this is particularly striking in the cave forms, but most of these species are basically similar to the form shown in fig. 1. The body outline is fusiform to oval, the antennae are four-segmented with a clear basal ring, the fourth abdominal segment is greater than three times the third, and the second thoracic segment is considerably longer than the third, frequently projecting forward so as to force the head down. The unguis and empodial appendages and reduced first thoracic segment are of the same basic type found throughout the family. The basal inner teeth of the unguis are often enlarged, and the empodial appendage may have a large wing tooth. The eyes are typically fewer than sixteen, and eyeless forms are very common. In spite of these common characteristics, examination of the clothing and male genital plates shows that *Sinella* belongs in the tribe Entomobryini while *Pseudosinella* falls in the tribe Lepidocyrtini.

During the past several years I have had an opportunity to study all of the Nearctic cave Entomobryinae, most of the Japanese forms, and a number of European species. It soon became clear that there are two types of characteristics: *cave dependent* and *cave independent*. The second type shows little evolutionary change within caves, and what changes do occur show no convergence and differ strikingly in degree within the different phyla. The cave dependent characteristics change markedly in cave life, all phyla showing some change, and convergence and parallelism are striking in most lines.

The existence of these two types of characteristic has greatly facilitated the study of the group. The cave independent characters are of wide usefulness only in the case of the chaetotaxy or scale covering of the head and body, the labial papillae, basic structure of the mucro (bidentate or falcate), absence or presence of a wing tooth on the empodial appendage, and the structure of male genital plate. Such characters are very useful in determining the phylum of an organism, but of little value in placing the form within a series. The cave dependent characteristics are more numerous, but only a few are widespread enough and show enough change to merit consid-

[1] Much of the work done upon this paper was made possible by NSF grant G-4563.

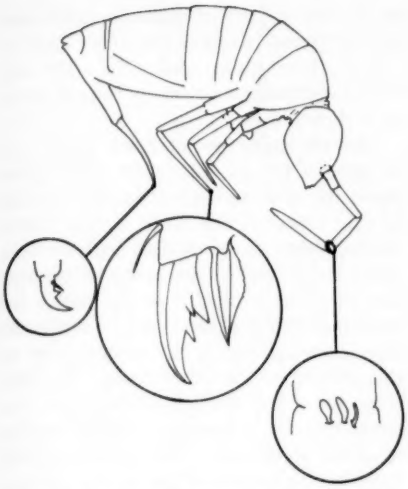

FIG. 1. Habitus of *Pseudosinella orba* to illustrate the location of various organs discussed in text. Lower circles: left—mucro; middle—tenent hair, unguis, and empodial appendage; right—apical organ of third antennal segment.

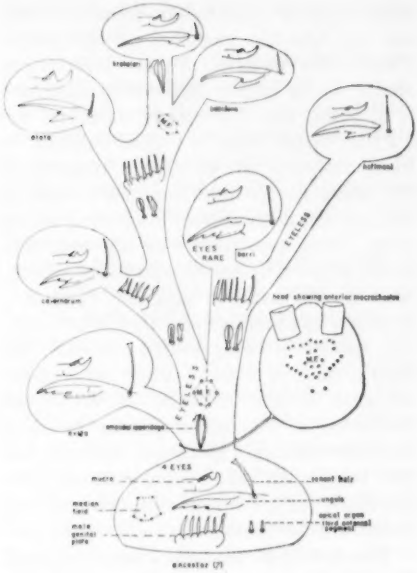

FIG. 2. Nearctic *Sinella*. Probable relationships among troglobite forms and the theoretical ancestor near *S. quadrioculata*.

eration here. Fig. 1 shows some of these characteristics. These characteristics are very useful for determining the position of a species within an evolutionary series, but convergence makes them very untrustworthy for determination of membership within a phylum.

The groups to be studied here fall into four major convenient categories: Japanese *Sinella* (Yosii, 1956), Nearctic *Sinella* (Christiansen, 1960a), Nearctic *Pseudosinella* (Christiansen, 1960b), and European *Pseudosinella* (Gisin, 1960). The genus *Entomobrya* has two true cave species: *E. troglodytes* from South Dakota and *E. pazaristei* from Europe. Both species show relatively little modification for cave life and will not be considered in detail here. A scattering of trogloxene or possibly troglophile species are found throughout the remaining genera of the subfamily. These show no evolution within caves and are therefore of no interest here.

In the following section I will attempt to outline the probable evolutionary patterns among the four major groups mentioned above. The schemes vary in reliability from good to poor. Nearctic *Sinella* are apparently a monophyletic group, limited in range and origin. In addition, they have an excellent range of cave independent and cave dependent characteristics, and I have been able to examine an adequate series of every species concerned. At the other extreme, European *Pseudosinella* represents a polyphyletic group, with wide ranges still largely uncollected. Most of the species can be known at present only from the literature and descriptions, which are often incomplete and sometimes erroneous. The other two groups lie between these extremes.

NEARCTIC *SINELLA*

Fig. 2 summarizes the relationships envisioned within the nearctic troglobite members of the genus *Sinella*. The basically similar structure of all the species concerned argues for a common origin, and in support of this they all share a peculiar

type of cephalic chaetotaxy, found nowhere else in Nearctic members of the genus. Clearly the member of this group most similar to the epigeic members of the genus is *S. avita*, and this species resembles the Pacific Coast form *S. quadrioculata* in many respects. If we accept ancestry of the whole group from some form near to this species, a number of interesting points arise. First, two species of the troglobite forms retain different aspects of the phenotypes of this putative ancestor. *S. avita* is generally the more similar but differs from this species in being totally eyeless. The typical form of *S. barri* differs from *quadrioculata* in most respects, but occasional specimens of the former species resemble the latter strikingly in unguis structure, and still rarer specimens of populations show the four-eyed condition, with the eyes represented by cornealess pigment spots.

The details of cephalic chaetotaxy, and the differing rates of development of cave dependent modifications, clearly separate *barri* and *hoffmani* from the remaining species. In addition to the characteristics shown in fig. 2, the two species mentioned above both show a peculiar condition of the macrochaetae at the base of the antenna which is lacking in other species, although approached by *avita*. The so-called "smooth" setae of the internal surface of the tibiotarsus go through an interesting series of changes. These cave independent structures have ciliations of moderate length in *S. quadrioculata*, while in *avita* the ciliations are noticeably longer. The greatest length of ciliation of the whole group is seen in *S. cavernarum*, in which there is a great deal of individual variation. In *basidens* and *krekeleri* there is gradual reduction of the ciliation, until in the last-named species the situation seen in *quadrioculata* is once more approached. In both *barri* and *hoffmani* the ciliations are much finer than in the other species. Another striking difference between the two branches of this group is in the rate of development of the peculiar paddle-shaped organs of the third antennal segment. These organs are in a fully developed state in both *barri* and *hoffmani*, whereas the other line develops them quite gradually. The antennae become more elongate in both series, as is shown in fig. 8.

The remaining evolutionary trends can be seen in fig. 2. The male genital plate shows the most varied pattern. Also of note is the forward migration of the lateral macrochaetae of the median cephalic field, which is followed by the eventual compression of the field itself. In the unguis the basal movement of the unpaired teeth with their eventual loss is accompanied by an enlargement of the basal teeth. The final stage shows a reduction of these two, accompanied by a peculiar basally swollen empodial appendage. Throughout all lines there is attenuation of the mucro, shortening and pointing of the tenent hair, increase of the antennal length, and general increase in body size.

The picture thus created is fairly clear in all details. As with all of the subsequent schemes, there is always the possibility of extinct additional links in the series, but the data presently available are quite consistent with complete pattern such as illustrated here. The most problematic question is the place of branching of the *barri* and *cavernarum* lines. The general evidence points to a common, now extinct, ancestor for both groups.

Nearctic *Pseudosinella*

The evolutionary pattern in this section is much more complex than that described above, with apparently three separate developments of troglobite forms, and a large number of isolated troglophile species.

The probable relationships of the various troglobite species are shown in figs. 3 and 4. Fig. 4 shows two of these forms, *P. espana* and *P. orba*, which are apparently single developments of troglophile forms. Both species are very similar to their putative troglophile ancestors, and in both the major changes are in cave dependent characteristics. Thus both species when compared to the troglophile forms show elonga-

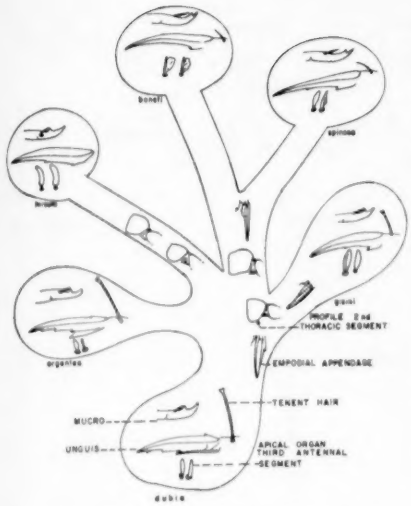

FIG. 3. Probable relationships among the forms of the main stream of cave evolution in Nearctic *Pseudosinella*. *P. argentea* and *P. dubia* are possible troglophiles, the others are troglobites.

tion of the antennae, as well as the changes illustrated in fig. 4.

The majority of the troglobite species belong to the series illustrated in fig. 3. These forms are strikingly separated from the other species of the Nearctic cave fauna by the total absence of macrochaetae in the median cephalic field. This phenomenon also serves to separate these forms from their European congeners.

These forms are also generally united by the fact that most show small internal ungual teeth and are generally limited to the caves of the SE United States. The only exception is *P. argentea*, which is also the only species with some evidence of non-troglobite habits. The exact relationships between this species and the remainder are very doubtful, but it appears to be most closely related to *P. dubia*, with numerous approaches to isolated populations of *P. hirsuta* either through hybridization or character displacement. In any case the

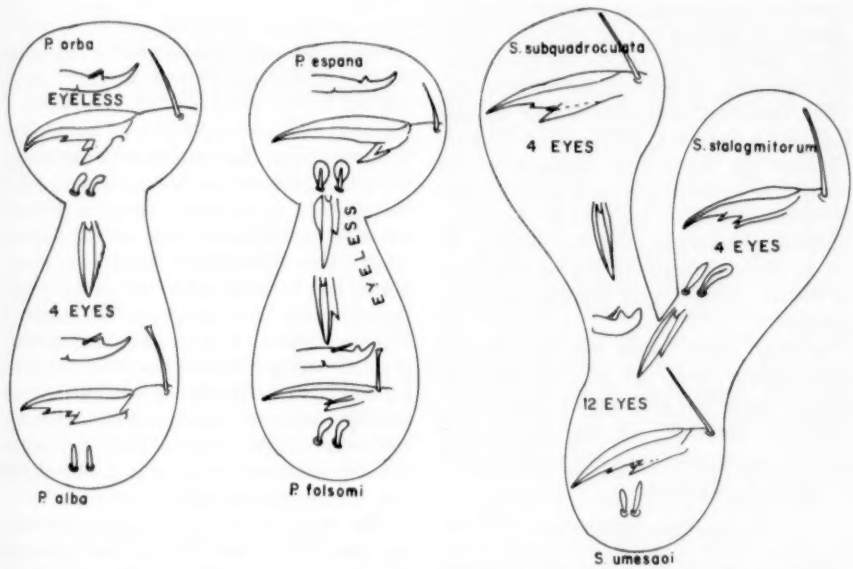

FIG. 4. Probable evolutionary relationships of the minor evolutionary lines in Nearctic *Pseudosinella* and Japanese *Sinella*. Left and center—Nearctic *Pseudosinella*; right—Japanese *Sinella*. *P. espana*, *P. orba*, *S. subquadrioculata* and *S. stalagmitorum* possible troglobites. The others are troglophiles.

species shows relatively little adaptation to cave life, has all the hallmarks of a true troglophile form, and is not a part of the main evolutionary stream of this group.

The remaining species present a much clearer evolutionary pattern. The unguis becomes more elongate and the teeth reduced with eventual loss of the median unpaired tooth in some specimens of *P. gisini* and all specimens of *P. boneti*. The empodial appendage becomes more elongate and develops a basal inner swelling, as happens in *P. espana* and *Sinella krekeleri*. The mucro becomes attenuate, but in the terminal species a peculiar apicalward migration of the median tooth occurs. This is seen in an early stage in *gisini*, occasional specimens of *spinosa*, and the majority of *boneti*. The expansion of the apical organ of the third antennal segment is much slower than in the genus *Sinella* and is not seen in full force until the final species, although occasional specimens of other species show this condition or some intermediate. Eyes are still present in *gisini* (4) and occasional specimens or populations of *hirsuta* (4) or (2), but in the latter species they usually are cornealess. The humping of the second thoracic segment, reduction in the tenent hair, elongation of the antennae, and increase in overall size are continuous throughout. In many species there are peculiar individual developments which may (as in the dental spines of *spinosa*) be cave independent, or (as in the chaetotaxy of the ventral head in *boneti*, *spinosa*, and *espana*) cave dependent.

In short, then, three entirely separate evolutionary lines appear in Nearctic *Pseudosinella*, but only one of these shows extensive speciation. In this line a clear pattern can be seen with one puzzling side branch in *P. argentea*.

Japanese *Sinella*

Figs. 4 and 5 represent the situation visualized in Japanese cave *Sinella*. Much of the evidence remains to be examined, but the schemes given probably represent

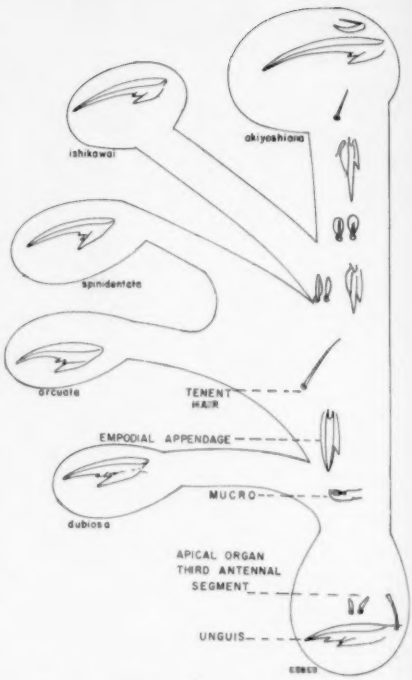

Fig. 5. Probable relationships of the main stream of evolution in Japanese *Sinella*. *S. caeca* troglophile, the others troglobites.

something near the evolutionary pattern in the group. There are two separate evolutionary lines, but only one has gone through extensive evolution in caves. The line in fig. 4 shows little change in the cave forms other than a slight expansion of the basal ungual tooth, a reduction in the number of eyes (from 5 to 2 per side), and the beginnings of expansion of the third antennal segment organ. It is quite possible that all are like *umesaoi* troglophile forms. All species are similar, with a bidentate mucro and chaetotaxy such as seen in the *dubia* line of Nearctic material.

The series in fig. 5 is far more interesting, and shows most of the developments present in Nearctic *Sinella*. The elongation of unguis, with enlargement then reduction of the basal tooth, the reduction of the median tooth, the elongation and basal

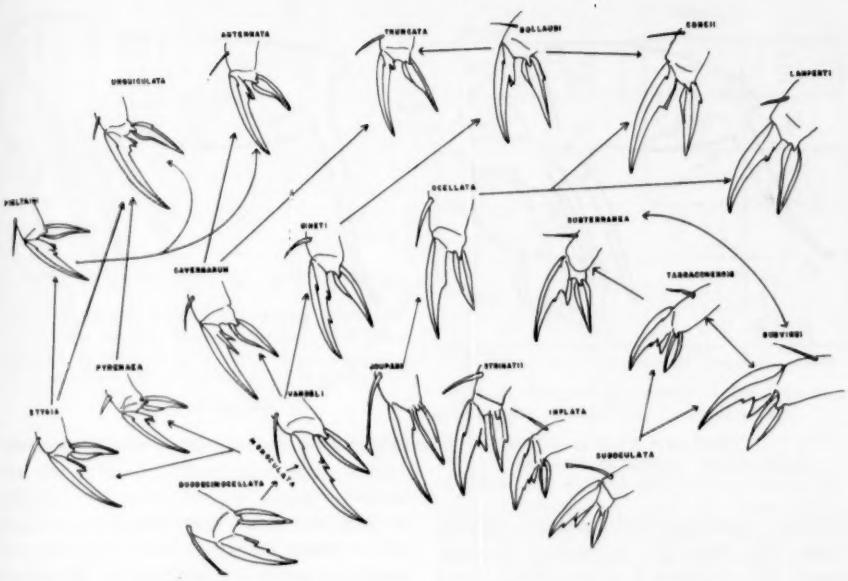

FIG. 6. Some possible relationships among European troglobite *Pseudosinella*. Three species are omitted due to lack of data.

swelling of the empodial appendage, the reduction of the tenent hair, the development of paddle-shaped third antennal segment organs, and the elongation of the antennae and mucro all occur. The putative ancestor of the group is *S. caeca*, and the basic chaetotaxy and sickle-shaped mucro in this species are maintained, along with an outer wing tooth on the empodial appendage, throughout the series. The exact location of the different members of the series is still in doubt, but the fact that *arcuata* and *spinidentata* on one hand and *akiyoshana* and *ishikawai* on the other intergrade in many characteristics makes the present scheme a likely one. In any case it is apparent that all the species concerned are closely related, and that they did not evolve in a simple linear series.

EUROPEAN *PSEUDOSINELLA*

The most complex and poorly known group of species is the European *Pseudosinella*. Most of the species are known only from descriptions, and on the surface it appears that while the majority are unusually local in distribution, a few species are extremely widespread. This may be the actual situation, but a thorough comparative study would probably uncover a great deal of synonymy and much wider distributions than presently indicated.

Some of the possible relationships among the troglobite species of this genus found in Europe are shown in fig. 6. Most of the possible evolutionary series here indicated are upon very shaky foundations, as available evidence is very incomplete. On the whole, the picture presented by the European species appears to be much more complex than any of the others, but generally follows the same pattern.

The total picture developed above is one of organs and organ systems evolving in a parallel or convergent fashion over a variety of lines in different regions, under the similar ecological conditions of cave life. Figs. 1–6 show most of the changes. The third antennal segment organ changes from peg-like to paddle-shaped. The tenent hair

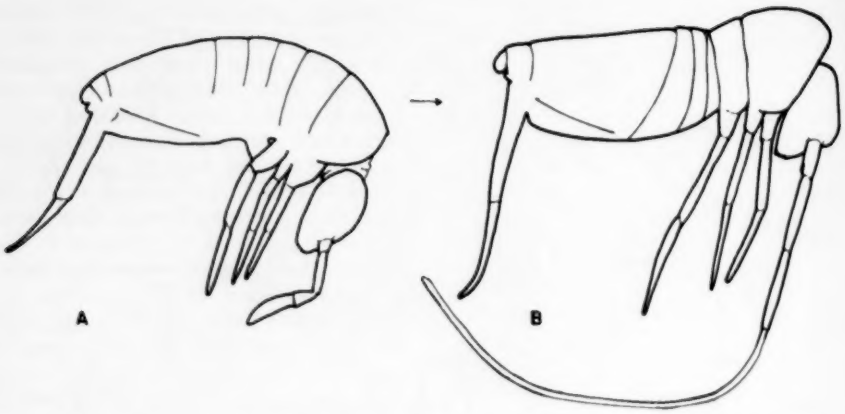

FIG. 7. Typical cave trend in habitus. A) Unadapted habitus in *Pseudosinella duodecimpunctata*. B) Highly adapted habitus in *P. boneti*.

becomes acuminate and strikingly shorter, while the empodial appendage becomes longer and develops a characteristic inner basal swelling. The mucro becomes more elongate, and in most forms the median tooth becomes relatively smaller, but in two lines (Japanese *Sinella* and Nearctic *Pseudosinella*) special trends can be seen in this organ. The unguis shows the greatest variety of structures, but these variations are largely in the intermediate stages of evolution, the early and late stages being quite uniform. The most highly evolved forms always have a very elongate unguis with a single basal tooth.

Fig. 7 illustrates the change in the habitus of these animals. This change is complicated by the fact that there is a considerable variety of body shapes in the presumed ancestral forms, and a corresponding variety in basic types. The sort of change illustrated here is the most common, and is similar to that in every group. The specimen at the left is *Pseudosinella duodecimpunctata*, a typical trogloxene form, the form at the right representing the apogee of cave habitus, found in *Pseudosinella boneti*. Although few cave forms are exactly like this, all highly evolved species show some change in this direction, and many are basically similar in structure.

In addition to these, there are a number of meristic changes such as increase in length of antennae, reduction of the second abdominal segment, and general increase in body size. Study of all of these is limited to species where considerable numbers of specimens can be examined, since most of the descriptions do not provide sufficient data to encompass either the growth or population variation in the various ratios. Series suitable for this type of study were available only for the Nearctic genera, and in these several gradual changes could be noted. The most striking concerns length of antennae as compared to length of the majority of the organs. Fig. 8 shows this change by comparing the most variable antennal segment with one of the most stable body organs—the head. An even more striking example is seen in *Pseudosinella* (Christiansen, 1960b). As is obvious from these charts, there is a gradual proportional lengthening of the antennae in all highly evolved Nearctic cave lines. Although exact comparison with the European and Japanese material is not possible, it is possible to see the outlines of the same process by summing the data available for a number of species.

In addition to all the changes listed above, there are a number of developments

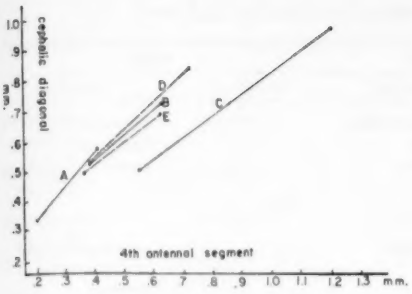

FIG. 8. Graph showing the change in the antennal–head ratio in Nearctic *Sinella*. A) *S. cavernarum*, *S. avita*, and *S. alata*; B) *S. basidens*; C) *S. krekeleri*; D) *S. barri*; E) *S. hoffmani*.

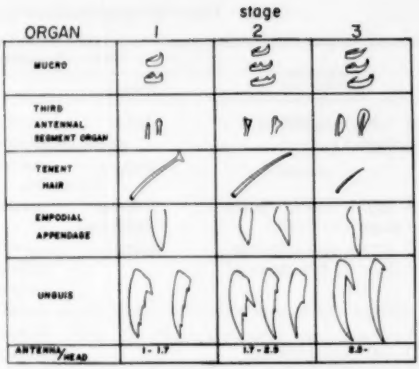

FIG. 9. Diagram to represent the three-step evolutionary scale in cave Entomobryinae.

of much more limited scope. Examples are the development of abundant ventral cephalic setae in several highly evolved species of *Pseudosinella*, and the gradual basal movement of the outer tooth of the empodial appendage in Japanese cave *Sinella*.

With all these cave dependent characteristics in mind it is possible to create an ideal series of organisms showing gradual change from a typical epigeic to highly evolved troglobite form. Almost every cave entomobryid can be readily placed in this series with a small amount of juggling to take care of the rare occasion when a species has primitive peg-like third antennal organs and a highly evolved unguis. Fig. 9 shows this from a slightly different viewpoint. It is possible to see these various characteristics in terms of a three-step scale of cave adaptation. If one characteristic falls in a given range, then the majority of the others also fall in this range. More than half of the organisms have one characteristic slightly out of line with the others, but very few have more than one and even fewer have them evenly split. This chart makes it possible to categorize all the species into seven steps: (A) those with all of group 1 characteristics, (B) those with mostly group 1 but one or more characteristics of group 2 etc., down through group (G) which has all group 3 characteristics. Table 1 shows all the cave species of these two genera categorized upon this basis. In stages (A) and (B) nine species are known troglophile forms. In stage (C) there are no definitely proven troglophiles, but *argentea* has been taken from graves and *dubiosa*, *monoculata*, and *stalagmitorum* are suspected to be troglophile forms. In stage (D), *cavernarum* has been taken from graves and reported (probably incorrectly) from other microcavernicole habitats. In stage (E), *P. immaculata* has been reported in caves and epigean localities, but it is quite likely that two species are involved. All the remaining species have been found only in caves. The distributions of the various species provide additional data. Trustworthy data for this section were available only for North American and Japanese species, and in order to get significant numbers, the three main evolutionary stages shown with (1), (2), and (3) were used. The range of each species was mapped by joining the extremes with straight lines and calculating the area occupied with a polar planimeter. Areas isolated by distances greater than 500 miles were measured separately and summed. Table 2 shows the results of these calculations. First stage forms are clearly limited in their cave distribution, even when they are very widely distributed as epigeic forms. A possible explanation for this is

TABLE 1. *Classification according to evolutionary stage*

Designation	*Pseudosinella*		*Sinella*	
	Nearctic	European	Nearctic	Japanese
A (all characteristics stage 1)	*dubia* *duodecimpunctata* *alba* *sexoculata*	*duodecimocellata* *suboculata*		*umesaoi* *curviseta* *caeca*
B (most characteristics stage 1)	*folsomi* *pettersoni*	*decipiens*	*avita*	
C (most characterstics stage 2, few stage 1)	*argentea*	*monoculata* *inflata* *joupani* *vandeli* *heteromurina*		*subquadrioculata* *stalagmitorum* *dubiosa*
D (all characteristics stage 2)		*strinatii* *subterranea* *aggtelekiensis* *ocellata* *subvirei*	*cavernarum* *barri*	*arcuata*
E (most characteristics stage 2, few stage 3)	*gisini* *hirsuta*	*virei* *stygia* *pieltaini* *tarraconensis*		*spinidentata*
F (most characteristics stage 3, few stage 2)	*orba* *spinosa* *espana*	*unguiculata* *lamperti* *pyrenea* *concii* *gineti* *cavernarum* *truncata*	*basidens* *hoffmani* *alata*	*ishikawai* *akiyoshana*
G (all characteristics stage 3)	*boneti*	*antennata* *sollaudi*	*krekeleri*	

their poor adaptation for cave existence. Most of these cave forms show slight structural change from typical epigeic types, and their occurrence in caves is usually peripheral and limited to regions not occupied by any more highly modified cave forms. The widespread nature of stage 2 forms may be explained by the fact that although they are clearly modified for cave life, and are able to compete very well in this habitat, they are not totally limited to caves in movement. Two species are able to live in microcavernicole habitats, and a number of others have been taken outside caves. Third stage forms are also limited in distribution, although much less strikingly so than stage 1 forms. It is clear that these forms are highly modified for cave life, and the fact that they often carve out exclusive territories in the heart of a heavily populated cave area indicates that they are very good competitors in this habitat. A probable explanation for their limited distribution is the nature of their cave modification. No species of this group has ever been reported outside a cave, and the nature of their modifications probably is of a limiting sort, as we shall show later. Another possible explanation for the lim-

TABLE 2. *Cave area occupied by different evolutionary stages*

	Total area occupied in square miles		
	Less than 1,000	1,000 to 10,000	More than 10,000
Evolutionary stage I	9 species	0	1 species
Evolutionary stage II	2 species	1 species	7 species
Evolutionary stage III	5 species	3 species	2 species

TABLE 3. *Number of cave localities for different evolutionary stages*

	Total number of localities			
	Less than 5	5 to 9	10 to 14	15 and more
Evolutionary stage I	8 species	2 species	0	0
Evolutionary stage II	3 species	0	3 species	3 species
Evolutionary stage III	4 species	2 species	2 species	2 species

ited distribution of these forms is that they are very recently evolved and are still in an early state of expansion. This probably plays some role in the process, and it certainly must in some situations; but it seems very unlikely that this is the major cause of the limitation, since it would require an amazing synchrony in invasion and evolution within the caves of North America and Japan. This synchrony would also have to include Europe, because the complete records we have from this area indicate a picture very similar to that in Japan and North America. Further evidence upon this matter is shown in table 3. Here we see the same three groupings with breakdown according to the number of localities from which the species has been reported. It is evident that while species of group 1 are limited both in range and occurrence, those of group three are limited only in range.

Many of the characteristics found in the second stage of cave evolution occur occasionally as preadaptations in ancestral epigeic or edaphic forms. Thus the enlarged basal ungual tooth and the absence of eyes and pigment are common characteristics of the genera *Sinella* and *Pseudosinella*, but when these are absent in the ancestral type they are quickly developed in the cave forms. In contrast to this, stage three modifications are developed only under cave conditions and therefore represent true troglobite organs.

Although these modifications are found only in cave forms in the genera mentioned above, they are often widespread in other taxonomic groups. A survey of the occurrence of such organs sheds considerable light upon their probable adaptive significance. Fig. 10 illustrates the occurrence of the elongate unguis in various groups. In each case the unguis in the upper row represents closely related forms showing the typical ungual structure of the genus concerned. A represents a member of the family Sminthuridae; C, E, and G represent three different tribes of the family Isotomidae; and I is the unique *Podura aquaticus*, the only member of the family Poduridae. These forms are varied in all respects save one—they are all aquatic inhabitants of the neuston, seldom found more than a few inches away from the surface of free-standing water. Further interesting evidence develops if we examine the kind of unguis and empodial appendage combination we usually find in primitive stage three forms. Fig. 11, D illustrates this form, which represents the condition seen in *Pseudosinella espana*. Again such structures are widespread, although by no means common. The remaining figures of the upper row illustrate such ungues in four additional genera of the Isotomidae and Entomobryidae. All have a common habitat—they are subaquatic. A, B, and E occur in the marine littoral zone, and C is found in old stream beds. Thus it is likely that the unguis modifications in cave Entomobryinae are developed first for movement over land and water surfaces and then primarily for water surface. The wet surfaces and standing pools so common in active caves afford ample opportunity for use of such modifications, and the fact that cave pools often act as natural Collembola traps indicates

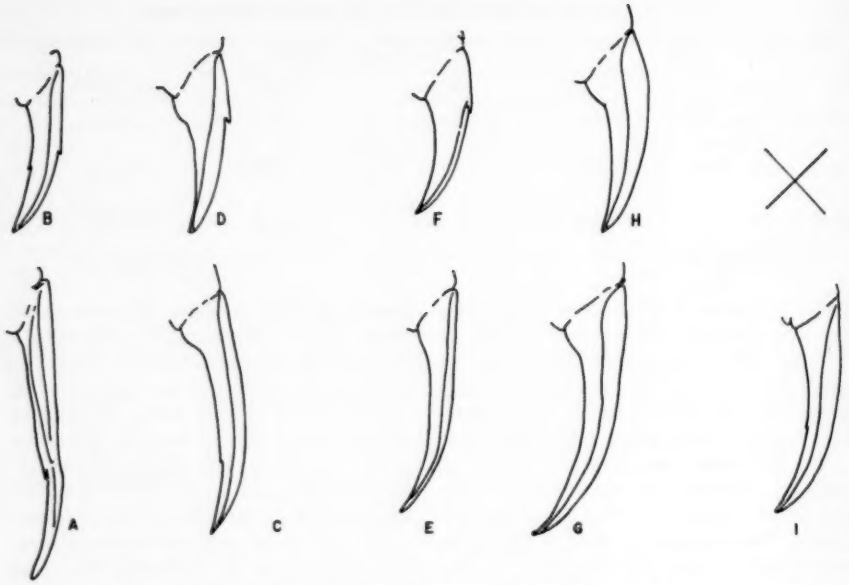

FIG. 10. Elongate ungues of various families and closely related short ungual forms. A) *Sminthurides aquaticus*; B) *S. schoetti*; C) *Proisotoma* (Hydroisotoma) *schaefferi*; D) *P. communa*; E) *Isotomurus retardatus*; F) *I. palustris*; G) *Ballistura schoetti*; H) *B. ewingi*; I) *Podura aquatica*.

a considerable selective advantage to such modification.

An entirely different set of characteristics is the peculiar habitus and elongate antennae and large size in the most highly evolved cave forms (fig. 7). In no other species are the antennae quite as long as here, but some elongation is prominent in all F and G forms. Fig. 11 shows that such developments are certainly not unique, but occur in several groups of epigeic Entomobryidae. The three forms illustrated belong to separate genera and two tribes, and many other forms belonging to several families have elongate ringed or subdivided antennae. All these forms have one thing in common—habitat. The huge majority of Collembola live in narrowly enclosed spaces, either in the cavities between soil particles, in leaf mold, or under bark. The forms mentioned above all have left these and live upon free surfaces, either on leaves, bark, or the soil surface. Those that invade this outer world always develop the peculair habitus shown. The caves furnish an almost exactly analogous situation, since the huge majority of the cave Entomobryinae live upon free surfaces. The functional significance of this is not clear, but in Entomobryidae these changes are associated with a considerable increase in the speed of escape and quickness of escape reactions, and free surfaces certainly place a premium upon this process. In hemiedaphic and edaphic forms such a system of protection would be of little value, and it is here that we see the development of spines, secretion, etc., rather than powerful mucrones and legs. If this is the major import of these modifications, then the elongate antennae may serve as an advance warning system. In any case, it seems certain that the development of elongate antennae and peculiar habitus in cave Entomobryinae is a response to the invasion of essentially free surfaces. Since epigeic *Pseudosinella* and *Sinella* are largely hemiedaphic and edaphic

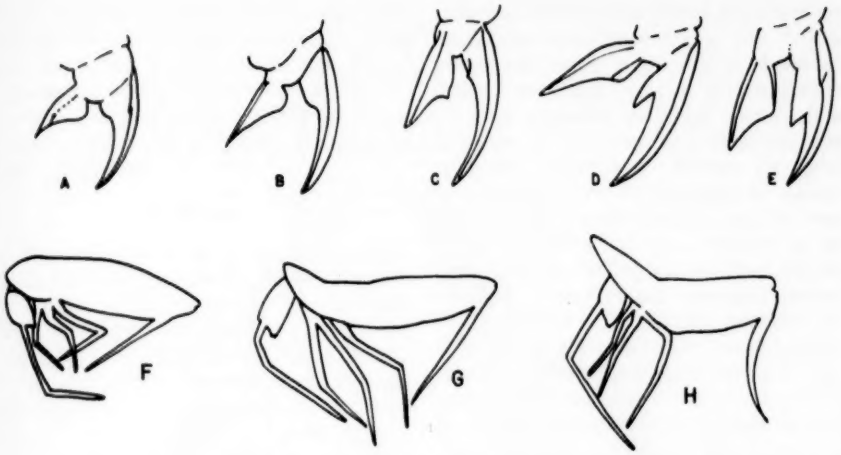

FIG. 11. Claws of littoral and cave forms and outline drawings of the habitus of various epigeic forms. A) *Isotoma boneti*; B) *Archisotoma besselsi*; C) *Lepidosira anomala*; D) *Pseudosinella espana*; E) *Mesentotoma laguna*; F) *Entomobrya bicolor*; G) *Lepidocyrtoides cucularis*; H) *Lepidocyrtus longithorax*.

forms, this certainly involves an alteration of selection.

The enlarged third antennal segment organ which is so universal in highly modified cave Entomobryinae occurs widely in other collembolan families (although nowhere else in the Entomobryidae). It is certainly not associated with aquatic life, and occurs in some whole families (Onychiuridae), in some as the major but not universal character (Oncopoduridae), or sometimes in a rare aberrant genus (*Isotomodes*). In all cases these animals are members of the euedaphon or deeper soil layers. Again the functional significance is not clear, but some possibilities may be adduced. First, it is unlikely that these organs are concerned with location of food. In the euedaphic zone food is sparse but widely distributed and probably can be located as well by random movements as by any other means. In addition, in forms that have a real problem in locating food (pollen feeders, algal feeders, carnivores, etc.) no such organs are developed. Their location makes it unlikely that they are used for locating predators or mates, but their extensive innervation makes it likely that they have an important sensory function. On the other hand, Agrell (1941) has shown, and personal observation has confirmed, that euedaphic forms are generally the forms most readily damaged by dryness, or temperature change. In this they agree with the troglobite forms, which are often so delicate that studies outside the cave can involve only the death throes of the animals. It is quite possible that these organs are associated with the need for remaining in a constant and relatively exact environment. The way this is accomplished is unclear, due to a welter of contradictory evidence concerning the responses of euedaphic forms to temperatures and humidity gradients.

A last characteristic is the loss of eyes and pigmentation. In cave Entomobryinae this is frequently preadaptive and always complete by the end of the second evolutionary stage, so it cannot be considered as a primarily cave modification. The evidence from other groups upon this question is massive, often conflicting, and usually confusing. No new insight can be gained from the material seen in this group except that it occurs in all groups of euedaphic

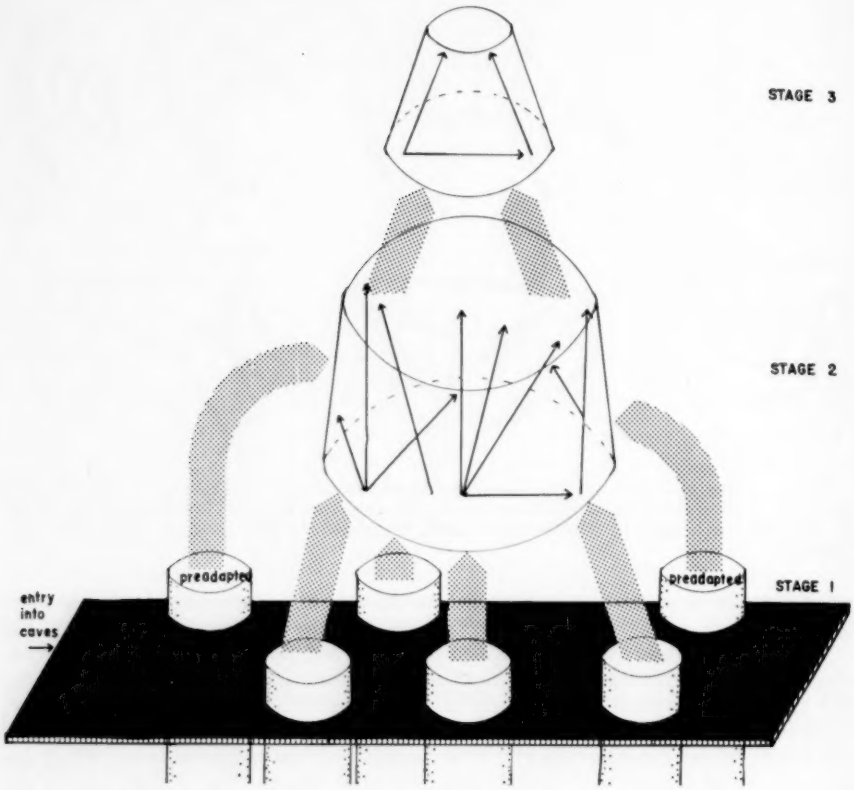

FIG. 12. Diagram showing evolutionary pathways in cave Entomobryinae.

Collembola. I feel that a final analysis of this problem must await experimental evidence from several groups of arthropods.

In conclusion, the chart in fig. 12 summarizes the view developed here concerning the evolution of cave Entomobryinae. Forms as they first enter cave life are preadapted to varying degrees. All such stage 1 forms are relatively poorly adapted to cave life, and remain limited and marginal as cave forms. If their invasion is successful they evolve a host of modifications to cave life and reach the second stage of cave evolution. Within this level a great diversity of evolutionary paths occurs, and although convergence and parallelism are common they are not compulsory. The general direction of evolution within this level is toward greater specialization, and is basically similar in all groups. These forms are not entirely limited to caves; some are able to invade other regions and many probably can live outside caves for a period. This combination of adaptation and mobility leads to wide distributions and numerical success. If these forms evolve far enough, they enter the third stage of evolution. Here the characteristics are strikingly similar in all groups and appear to be close adaptations to the requirements of cave life. A small amount of divergence occurs in the early stages, but evolution within the stage is convergent and the end forms are often astonishingly similar. The third stage forms are so highly modified for cave life that they rarely

move beyond a single cave system. Although the modifications occurring within and before this stage are in no way degenerative, but represent positive adaptations to cave life, the net result is that the forms are trapped by the excellence of their own cave adaptations.

Summary

The evolution of the holarctic cave Collembola of the subfamily Entomobryinae is analyzed. A number of evolutionary lines are shown, involving the two polyphyletic genera *Pseudosinella* and *Sinella* in Japan, North America, and Europe. Characteristics of these animals are of two types: *cave dependent* (responding rapidly to the cave environment) and *cave independent* (showing no effect of the cave environment). The evolution of the cave organisms is divided into three stages. The first stage represents the level of adaptation normally found in troglophile organisms, although some troglobites are found in the stage. All such forms are very limited in their cave distribution, and occupy a few caves around the periphery of major cave habitats. The second stage of evolution shows more cave adaptation, but the evolutionary pathways are varied, with convergence, parallelism, and divergence common. Most forms within this stage are troglobites, but there are a few troglophiles, and many of the organs characteristic of the stage occur as preadaptations in some putative ancestral forms. The members of this stage are abundant and relatively widespread. Within the third evolutionary stage convergence is universal, and the organs characteristic of these forms are limited to cave members of the family. Forms falling within this stage are relatively abundant but extremely limited in distribution. The probable adaptive significance of several organ modifications characteristic of troglobite forms is discussed, and these structures are interpreted as positive adaptations to the new requirements of cave life which have the final result of closely limiting the animals to the cave environment.

Literature Cited

Christiansen, K. 1960a. The genus *Sinella*, Brook (Collembola: Entomobryidae) in Nearctic caves. Ann. Ent. Soc. Amer., **53**: 481–491.

——. 1960b. The genus *Pseudosinella* (Collembola Entomobryidae) in United States caves. Psyche, **53**: 1–25.

Gisin, H. 1960. Collembolenfauna Europas, Geneva.

Yosii, R. 1956. Monographie zur Höllencollembolen Japans. Contrib. Biol. Lab. Kyoto Univ., **3**: 1–103.

REPRODUCTIVE MECHANISM OF UNISEXUAL AND BISEXUAL STRAINS OF THE VIVIPAROUS FISH *POECILIOPSIS*[1]

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The fishes of the order Cyprinodontiformes have evolved a variety of systems of sex-determination, some of which are unique among vertebrate animals. One such anomalous condition was recently reported by Miller and Schultz (1959) in the viviparous genus *Poeciliopsis* Regan, wherein certain strains gave birth to only female offspring. *Poeciliopsis* comprises approximately 16 species that range from southern Arizona to Colombia, with at least two endemics in the Atlantic drainage of the Isthmus of Tehuantepec (fig. 1). Western Mexico harbors the largest number of species.

This study deals principally with two species of *Poeciliopsis* tentatively assigned to the species group *Leptorhaphis* Regan (Miller, 1960). Since neither species has been described,[2] they are here assigned the symbolic designations "C" and "F." *Poeciliopsis* "C" is found in southern Sonora and northern Sinaloa, where it is confined to the Mocorito, Sinaloa, and Fuerte river drainages. The southern limit of "F" is less than 50 miles to the north; it extends from the Rio Mayo northward to the Yaqui, Matape, Sonora, and Concepción rivers in Sonora.

From preserved material collected within the range of strain "F," Miller was able to identify females with two types of dentition. One of these is considered to be the normal type in that the minute inner teeth are like those of the male. They consist of one or two uneven rows that follow the heart-shaped curvature of the lips and the outer row of teeth. The second type of dentition is characteristic of an aberrant strain of "F" females which are designated as "F_x." The inner teeth of this strain consist of a fine sandpaper-like patch on either side of the midline. No other consistent morphological differences have been found between the two strains, yet reproductively they are radically different. Matings of "F" females having normal dentition to "F" males result in young of both sexes, but the "F_x" females with aberrant dentition mated to the same male produce only female progeny.

The same aberrant dental character was later found in samples of strain "C." In these, however, dentition proved to be less reliable for distinguishing the unisexual strain, designated "C_x," from the normal females. Offspring from "C_x" individuals showed a spectrum of variation from the nearly normal to the aberrant types of dentition. These progeny were nevertheless all females, as were the succeeding generations.

This "all-female" phenomenon appears to be similar to that discovered by Hubbs and Hubbs (1932) in *Mollienesia formosa*, a member of the same family as *Poeciliopsis*.

It is the purpose of this study to find the sex-determining mechanism responsible for unisexuality in *Poeciliopsis*. Aspects of the reproductive biology and genetics of both the bisexual and the unisexual types, therefore, have been studied and comparisons have been made with other animals known to have unusual sex-ratios. The following mechanisms have been considered: lethal systems, polyploidy, parthenogenesis, gynogenesis, sex-inversion, cytoplasmic in-

[1] Part of a thesis submitted to The University of Michigan in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1960. Cost of extra pages paid for by the author.

[2] Since this was written, the species here referred to "C" and "C_x" has been described as *Poeciliopsis lucida*; the one designated "F" and "F_x" may be identical with *P. occidentalis* (Miller, 1960).

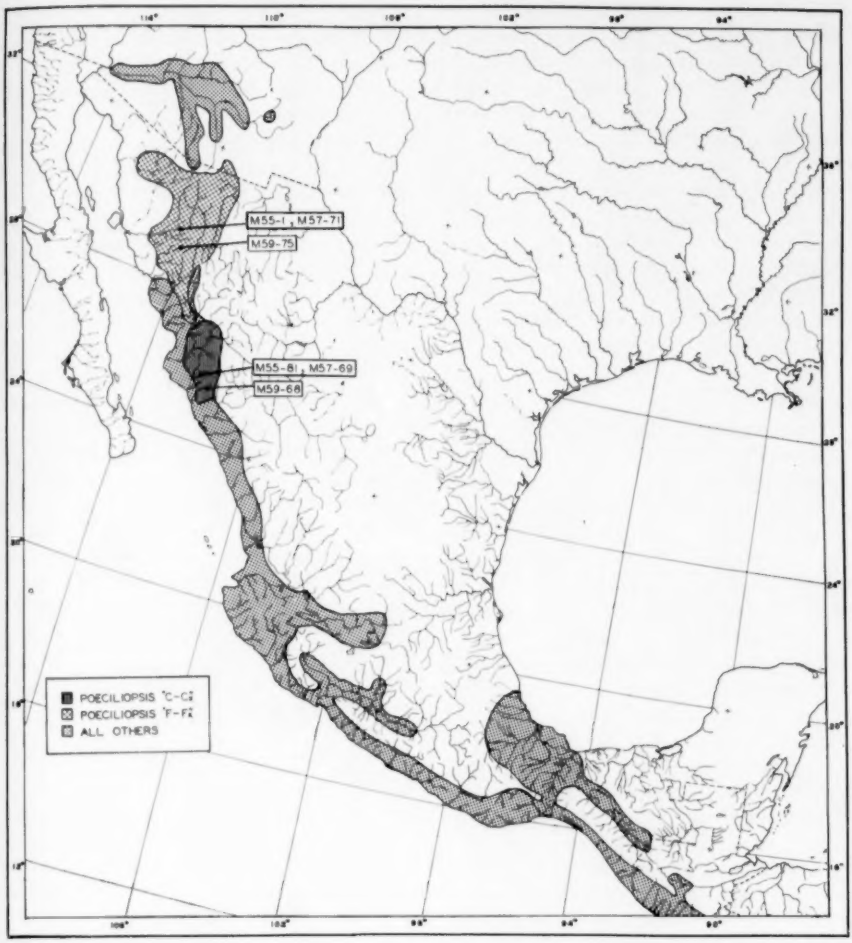

FIG. 1. Distribution of *Poeciliopsis* in part of North America, showing localities from which live stocks were obtained.

heritance, unbalanced autosomal system, and selective maturation.

MATERIALS AND METHODS

Live stocks used in this investigation were collected in northwestern Mexico during expeditions by Miller and Greenbank in 1955, Miller and Miller in 1957, and Miller and Schultz in 1959. The localities from which they were obtained are indicated in fig. 1 by collecting station numbers.

Specimens to be used for genetic analysis or to be dissected for gonadal studies were fixed in 10% formalin and preserved in 70% ethyl alcohol. Examination of live material was facilitated by the use of an anesthetic, MS-222 (Tricaine methanesulfonate). Immature fish used in histological studies were killed and fixed in

Bouin's solution and preserved in 70% alcohol. The entire fish was embedded in paraffin and sections were cut at 10 microns. These were stained in Ehrlich's haematoxylin and counterstained with eosin, and were examined at magnifications of 430x and 980x. Cytological preparations were made from embryos one or two days old and from testes of adult males. The tissue was killed and fixed in Newcomer's solution, stained in aceto-carmine and squashed between cover slip and slide. All fish measurements are the total length in mm estimated to the nearest tenth.

Reproductive Biology

Insemination.—*Poeciliopsis*, being a viviparous fish, has internal fertilization. Spermatophores or sperm packets are placed in the genital pore of the female by means of the male's modified anal fin, the gonopodium. Here, the packets break up, releasing the spermatozoa. These migrate up the gonaduct to the ovary where they remain viable for months. As a result of sperm storage, *Poeciliopsis* females may produce as many as ten broods of young after they have been isolated from their mates.

Ovarian Cycle.—Most viviparous fishes have only one stage of embryos in the ovary at a time: the birth of these must take place before the next series of ova is fertilized. *Poeciliopsis*, however, has superfetation, wherein two or more stages of embryos develop simultaneously (Turner, 1937).

From the brood records and from the examination of more than 400 females, a generalized sequence of events occurring within the ovary has been reconstructed. Except for minor deviations it seems applicable to all species of *Poeciliopsis*.

Ovaries of submature females contain several strings of tiny, white oocytes, .25 to .30 mm in diameter. As the female matures, one or two of these oocytes begin to accumulate yolk, which gives them an amber color. The nucleus is centrally located in these submature eggs; but upon completion of yolk deposition, it migrates to the periphery, where maturation division occurs.

After this series of eggs is fertilized, yolk accumulates in a second series. By the time the first embryos are 7 to 10 days old and have reached the halfway point in their development, the second series of ova is fertilized. In strains "C" and "C_x" the blastula or streak stage of a third brood of embryos is sometimes found before the birth of the first or oldest stage of embryos, but in "F" and "F_x" no more than two stages of embryos occur at one time.

If sperm are not available to fertilize the newly matured ova the condition does not remain static but degeneration of the ova ensues. Degenerating ova and embryos are common in females living under suboptimum conditions or in those used in certain hybrid crosses. Frequently one to several ova of a clutch fail to become fertilized or an embryo dies in the course of its development. The age of these can be correlated with the brood of embryos of which they normally would have been a part. Degenerating elements in the ovary can be recognized for more than two weeks after their death.

In dealing with unisexual animals, one of the concerns is whether or not the one sex dies before maturity. A more complete evaluation of lethals as they relate to the unisexual strains of *Poeciliopsis* will be considered later, but certain aspects that involve the ovary are treated appropriately here.

In a reproductive system such as that of viviparous fishes, a lethal mechanism acting during the intraovarian cycle would reveal itself by the presence of dead embryos, eggs, or oocytes. In "C_x" and "F_x," a sufficient number of failures to account for unisexuality might be expected at any of the following stages of the ovarian cycle: (1) maturation division; (2) fertilization; (3) early cleavage; and (4) advanced embryos. In "C" and "F" no more than a few prenatal deaths theoretically should occur.

From the 38 "C" and "C_x" females dis-

TABLE 1. *Comparison of intraovarian mortality between strains "C" and "C_x"*

Strain of female	Number dissected	Ova and embryos observed	Per cent of mortality
C	11	102	21.7
C_x	27	319	5.6

TABLE 2. *Minimum and average brood interval in days of strains "C," "C_x," "F," and "F_x"*

Strain of female	Sample size	Minimum brood interval	Average brood interval
P. C	182	4	11.5
P. C_x	124	7	11.7
P. F	26	5	13.8
P. F_x	76	9	13.2

sected for this phase of the study, only a few dead embryos could be found. The degenerating elements observed were for the most part identified as ova. Many of these, however, were undoubtedly early embryos undetected because of their small size and degenerate condition. When the total intraovarian mortality of "C" and "C_x" was compared (table 1), the unisexual strain actually had fewer degenerating elements (5.6%) than did the bisexual strain (21.7%). A study of the aquarium history of each female revealed that all of those containing more than one dead ovum or embryo had been subjected to suboptimal rearing conditions such as inadequate illumination or overcrowding. Since strain "C" is in general less hardy than "C_x," the higher mortality rate in the ovaries of these fish is accountable. When individuals of both strains are maintained under ideal conditions, intraovarian failures are almost negligible. It must, therefore, be assumed that if differential mortality is responsible for unisexuality in this genus, it takes place during a later stage of development.

Brood Intervals and Brood Sizes.—Development of superfetation in poeciliid fishes has significantly reduced brood intervals and brood sizes. In *Lebistes* and similar forms that lack superfetation, as many as 75 young are born at intervals of 22 to 30 days, whereas species with two or three stages of embryos seldom have more than 25 young per brood. These are born at intervals of 7 to 14 days.

Normally there is little fluctuation in the brood interval of healthy *Poeciliopsis* females; but in compiling extensive data for comparing species or strains, some distorting features must be taken into account. Occasionally a single young from a brood is retained in the ovary and is not born until one or two days after the others. Females in poor condition have irregular intervals, but even healthy individuals experience interruption or "rest periods" after a long sequence of births. Although more typical of winter months, long brood intervals also may occur during the summer. The above irregularities were disposed of by considering only those intervals that fell between 4 and 20 days (table 2). Comparison of the average brood intervals of the four strains of females revealed no significant difference between the 11.5 day interval of "C" and the 11.7 day interval of "C_x," nor was there any difference between the 13.8 and 13.2 day intervals of "F" and "F_x." The occasional presence of three stages of embryos in the ovaries of "C" and "C_x" is reflected in the shorter brood intervals of these two strains.

Brood size data are difficult to evaluate. Not only does the number of young in broods from a single female sometimes vary for no apparent reason, but environmental factors also affect the reproductive condition of the fish. In *Poeciliopsis*, inadequate illumination, even during early life, may reduce the reproductive capacity of females. Artificial light sufficient to produce a plankton bloom does not compare with natural light for rearing *Poeciliopsis* young. Overcrowding and peck-order dominance result in stunted fish which, upon reaching adulthood, may have reduced fecundity. These factors are so complex as to constitute a separate study and will not be pursued here.

In order to obtain at least some notion of the reproductive potential of *Poeciliop-*

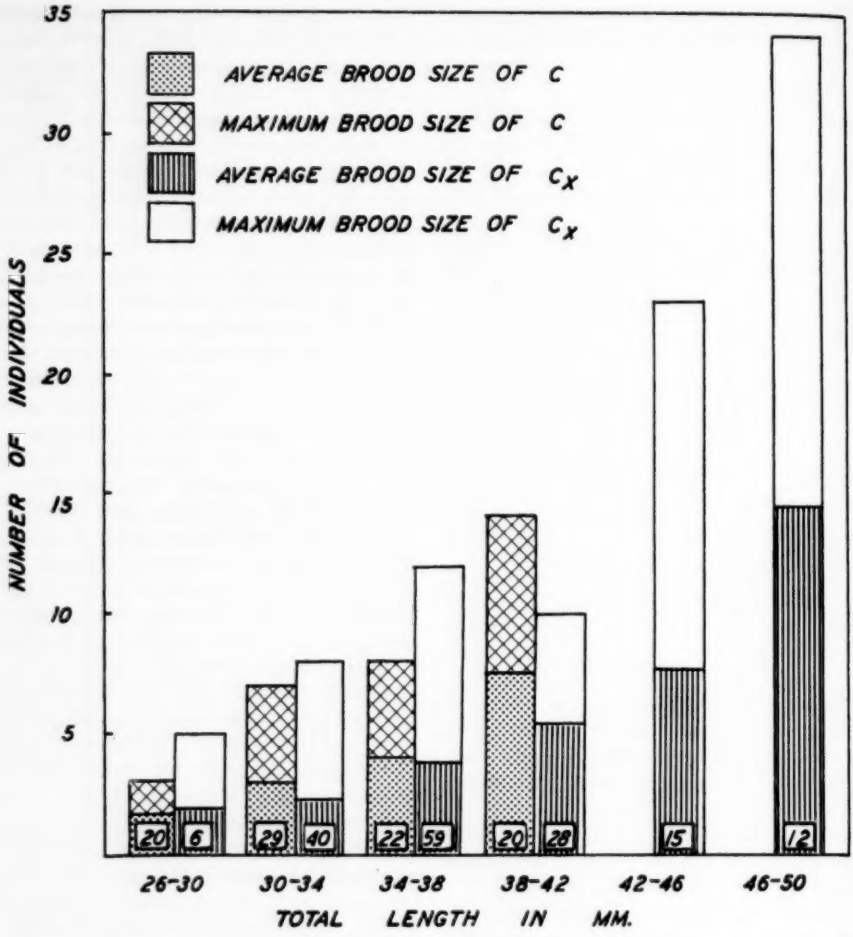

FIG. 2. Average and maximum brood sizes of "C" and "C_x" females in relation to the total length of the female. The sample size of each class is indicated in the square on each bar.

sis, females of each type were anesthetized and measured after the birth of young. The average and maximum brood for females of various size classes are presented in figures 2 and 3. Although certain size classes are poorly represented, particularly for "F" and "F_x," several interesting tendencies are apparent. In comparing strains "C" and "C_x," the expected trend of larger females to have larger broods is evident. The two strains behave differently, however, in that "C" reaches its maximum brood size and stops reproducing at about 41 mm, whereas "C_x" continues to have young until about 50 mm long. In these size ranges "C_x" has produced broods with far more young than any other species of *Poeciliopsis* reared in this laboratory. Four such broods contained 34, 23, 21, and 19 offspring. Only "C" has approached this record, with broods of 16, 14, 12, and 12. Since only two size classes of "F" are rep-

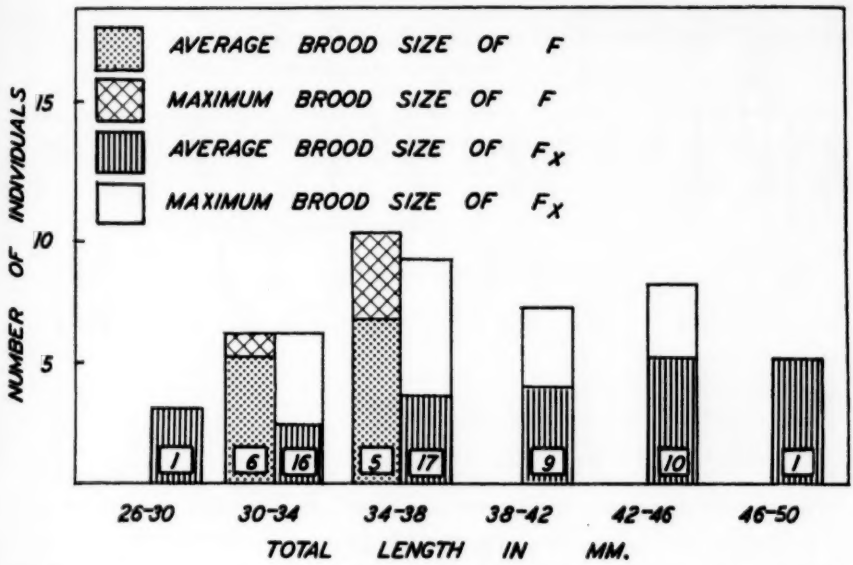

FIG. 3. Average and maximum brood size of "F" and "F_x" females in relation to the total length of the female. The sample size of each class is indicated in the square on each bar.

resented, all that can be said of the reproductive potential of this species is that the maximum brood consisted of 10 young—these by a 36.4 mm female (fig. 3). Although the data for "F_x" are somewhat scanty, this strain apparently produces fewer young in the laboratory than either "C" or "C_x." "F_x" females do, however, attain nearly the same maximum length as the "C_x" females. It is also possible that "F_x" produces fewer young than "F," but more information is required to clarify this point.

Maturation and Senescence.—The attainment of sexual maturity in *Poeciliopsis* females is not accompanied by apparent external changes. Therefore, dissection was required in order to determine the age or size at which females become adults. Ovaries from aquarium-reared individuals of various ages and sizes were examined. Those with at least one mature or degenerating ovum were considered fully developed, whereas the presence of only oocytes, whether or not they contained yolk, was interpreted as evidence of immaturity.

The various species and strains of *Poeciliopsis* differ in the size and age at which they mature. Of the four types considered here, "C" is the smallest. Females of this strain have mature ova when 20 to 22 mm long and at an age of 49 days. The youngest to reproduce were 69 days old and 21 to 23 mm long. Strain "C_x" matures at 21 to 24 mm and at a minimum age of 70 days—20 days older than "C."

Females of strains "F" and "F_x" matured at a minimum size of 23 mm and at an age of more than two months; no young were born prior to the attainment of 29.9 mm. The variations and discrepancies among individuals, however, indicate that in these strains, particularly in "F," factors influencing gonad development are more complex than those involved in either "C" or "C_x." Five of the "F" females examined exceeded 25 mm; yet only one of these contained ova. Certainly their size should have been sufficient for gonadal

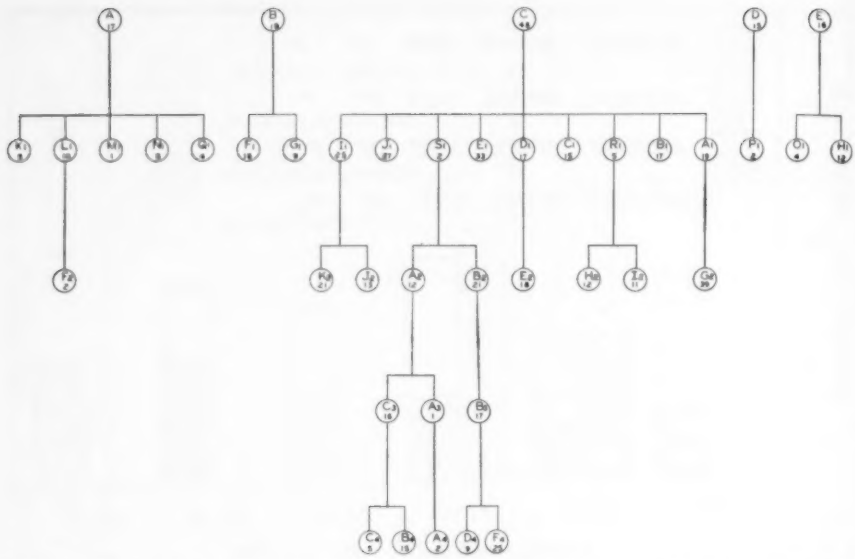

FIG. 4. Descendants of five wild "C_x" females from M57-69. Letter = female designation; subscript = laboratory generation; number = offspring from each female that were raised to maturity. All young that matured were females.

maturation. There is some evidence that females born during the summer do not mature until early spring of the following year.

Strains "C" and "C_x" differ not only in the size and length at which they mature but also in the maximum length attained. Females of "C" reach their maximum length at about 43 mm, whereas "C_x" females not uncommonly exceed this by more than 5 mm. "F" females were smaller than those of the other three strains. Their condition, however, probably reflected the effects of aquarium life, since specimens collected in nature and placed in the aquarium had lengths of 40 to 43 mm, approaching the maximum for "F_x." The largest of the "F_x" females had total lengths of about 46 mm.

Females of most species of *Poeciliopsis* reared under optimum conditions live for about a year and a half; 678 days was the maximum age attained by any individual. No great difference was found in the longevity of the four strains.

The number of days required for males to mature, as evidenced by gonopodial development, varied considerably among individuals and species. "C" males on the average reached adulthood in approximately half the time required for "F"—68 versus 142. Once mature, the males stop growing. No significant change in length could be demonstrated over a period of five to nine months for five adult males of *Poeciliopsis* "C." The total life span for males of each species is about one year, but a few individuals have lived more than a year and a half.

EXPERIMENTAL RESULTS

Intraspecific Matings and Sex-Ratios of Offspring.—Although it is known that certain *Poeciliopsis* females give birth only to female offspring whereas others, mated to the same male, produce young of both sexes, several questions arise regarding the nature of such reproduction: (1) Do the unisexual strains consistently produce female offspring or does an occasional male

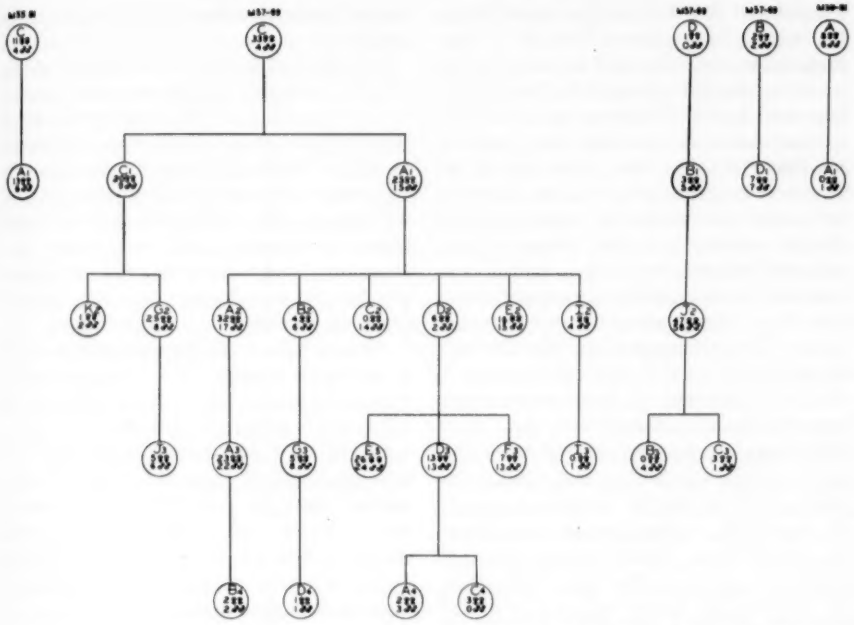

FIG. 5. Descendants of five wild "C" females. Letter = female designation; subscript = laboratory generation; number = offspring from each female that were raised to maturity.

result? (2) In broods from unisexual mothers, do bisexual type females ever arise? (3) Do bisexual females sometimes give birth to the unisexual type offspring? (4) What is the sex-ratio among progeny from the bisexual parents?

In an attempt to answer these questions, females of all four strains were mated and the resulting young raised to maturity. The hardiness of "C" and "C_x" made possible the backcrossing, outcrossing, and inbreeding of various lines, but matings of "F" and "F_x" were somewhat limited by the reduced fecundity of "F" under aquarium conditions.

From the live material collected in 1957, five females proved to be of the "C_x" strain. These and their descendants (fig. 4) gave birth to 923 young. After 50 died of natural causes and 276 were either discarded or preserved, 597 attained maturity; not one of these was a male. Although offspring were produced by 41 different parents, for only those "C_x" females which had nine or more female offspring can randomness be discarded as a factor in the unisexuality of the progeny. From the five original "C_x" females, 25 descendants fulfilled these requirements, whereas in not a single one of the 36 F_1–F_4 generations could a transfer to the bisexual type be demonstrated.

The same 1957 collection from which the "C_x" aquarium stock was derived contained three females that ultimately were shown to represent the bisexual strain. Two other females, one from the 1955 collection and one from the 1959 collection, were also of the "C" type. From these five females and their descendants, 744 young were born, some of which constituted an F_5 generation (fig. 5). The number of these offspring that reached maturity was 592 (an additional 97 died of natural causes and 55 were discarded or preserved as young). Of those that matured, 313 were

females and 279 were males—essentially a 1:1 ratio (Chi-square = 1.95, $P > 10$). From the original five wild females, 28 representing the F_1 through F_4 generations were mated. All but one of these produced at least some males and, therefore, must be considered bisexual. Since from the one female only three offspring reached maturity, she cannot be considered unisexual even though no males developed. When the sex-ratio of offspring from each female was analyzed, it was seen that significant deviations from the expected 1:1 ratio occasionally occur; examples are M57-69 ♀G_2 (with 25♀♀:8♂♂) and M57-69 ♀C_2 (with 0♀♀:14♂♂). A number of authors (particularly Krumholz, 1948) have indicated that among the viviparous fishes males are less hardy than are females. In explanation, Essenberg (1923) suggested that the higher metabolic rate of males is responsible. In strain "C," although a 1:1 ratio was demonstrated, there is still an apparent tendency for more females to reach maturity than males. It is also possible that males are more delicate than females during the period between birth and the attainment of adulthood. Since 14.2 per cent of the offspring from "C" females died before they matured and only 7.7 per cent of the "C_x" young died during the same period, differential mortality might account for the deviation of the ♀G_2 progeny from a 1:1 ratio, and also cause the total number of "C" offspring to comprise slightly more females than males.

The exceptional sex-ratio of ♀C_2, however, cannot be accounted for on the basis of differential mortality or chance. Since the probability of obtaining 14 male offspring by chance alone is 1 in 16,224 it might be expected that one parent or the other carried genetic factors that were strongly male-determining. The pedigree of the parents involved in this event, however, did not suggest this. A series of matings were conducted to determine whether or not this maleness was genetically transmittable, but neither by inbreeding nor backcrossing were abnormal sex-ratios again produced.

Information derived from matings of the "F–F_x" group is similar to that of the "C–C_x" group. All "F_x" individuals were derived from three wild females collected in 1955. These and their 11 F_1- through F_4-generation female progeny gave birth to 453 young, of which 290 females were reared to maturity (112 were either preserved or discarded and 51 died of natural causes). As was true of "C_x," not a single male was produced.

The situation in the bisexual strain, "F," is similar to that of "C." Offspring were obtained from 17 wild females collected in 1955, 1957, and 1959. Of the 243 young born, 70 died of natural causes and 45 were discarded or preserved. The 128 that reached maturity consisted of 75 females and 53 males. Here again, as in "C," the deviation from a 1:1 ratio is not significant at the .05 level; yet, some differential mortality is strongly suggested.

It is now possible to answer within certain limits the questions posed earlier regarding the nature of unisexual and bisexual reproduction in *Poeciliopsis*. In view of the fact that no males occurred among 887 offspring of the two unisexual strains, the likelihood of such an event taking place is fairly remote. It is furthermore unlikely that females of either the bisexual or unisexual strains ever produce offspring of the other type. The sex-ratio of the bisexual strains is probably about 1:1 at birth, and although differential mortality results in a few more females reaching adulthood than males, the deviation from a 1:1 ratio is not sufficient to call for the postulation of a genetic system.

Early Gonad and Sex-Inversion.—The occurrence of unbalanced sex-ratios among cyprinodont fishes has been subject to numerous investigations. Notable among these is the work of Essenberg (1923), who showed that the preponderance of males in aquarium stocks of *Xiphophorus helleri* was a result of sex-inversion. Conceivably, inversion of sex could be carried suffi-

ciently far to result in the unisexual strains of *Poeciliopsis*. Here, however, the inversion must be reciprocal to that of *Xiphophorus*, so that all individuals genetically oriented to maleness are converted to females. If such a reversal does take place, examination of gonads from a sufficient number of "C_x" or "F_x" young at the time that sex is first distinguishable should reveal individuals with rudiments of testes. Because "C" and "C_x" young were more readily available, they were chosen for this study. Offspring of different ages were sectioned and their gonads examined.

The gonads of *Poeciliopsis* "C_x" young killed at birth are sexually undifferentiated. They are widely paired and are suspended beneath the swimbladder in the peritoneal sac that later becomes the permanent wall of the gland. Internally they are characterized by two cell types: (1) the small stroma cell that is indistinguishable from peritoneal cells and has an irregular, darkly staining nucleus, and (2) a series of larger cells representing the initial stages of transformation from the stroma to the primordial germ cell. In the course of this transformation, the nucleus and cytoplasm of the stroma cell increase in size, the chromatin becomes coarse and starts to clump and the once irregularly shaped nucleus rounds out, and loses its affinity for stain. Both the stroma cells and the series of transforming germ cells are evenly distributed throughout the indifferent gonad.

By the sixth day several changes have taken place and it is possible to distinguish the sexes cytologically. In females, the two halves of the ovary have begun to fuse anteriorly. The number of transforming germ cells has significantly increased, and some have more than doubled in size. The nuclei of the largest have lost their affinity for stain and, except for the strands of chromatin, are now lighter than the cytoplasm. The germ cells of the early ovary remain evenly distributed throughout the structure; but most of the stroma cells have become pressed about them in the initial formation of the follicle. By the eighth day the ovary is filled with germ cells of various sizes; the two halves of the gonad are almost completely fused and its shape has become quite irregular.

Four of the eight "C" young that were sectioned were males. Three characteristics render the early testis distinct from the early ovary: (1) the germ cells are all approximately the same size, (2) most of the stroma cells are located medially, whereas the germ cells are pushed laterally, and, (3) some of the stroma cells have clustered together at the center of the testis in the formation of sperm ducts.

Having determined the differences in structure of the early testis and the early ovary, 13 "C_x" females, 6 days old, were examined for evidence of maleness. The mother of these young was an F_1 female that had produced 33 other offspring, all of which matured as females. All 13 of the sectioned gonads were unmistakably female. Since the probability is 1 in 8,112 that 13 females would be selected by chance alone, it is safe to conclude that at the time sex is first established all "C_x" young are females. Hence, the premise that sex-inversion is responsible for broods of all-female offspring has no histological support. The genetic evidence, considered later, further refutes this possibility.

Cytological Results.—Chromosome numbers have thus far been determined for 27 species of cyprinodont fishes (Wickbom, 1943; Makino, 1951; and Öztan, 1954). With few exceptions the haploid number is 23 or 24 and the diploid, 46 or 48. In all of these studies the small size of the chromosomes prevented detailed morphological analysis; consequently, little is known beyond the fact that most are rod-shaped, with terminal centromeres.

In the present study counts were made from spermatogonial divisions of strains "C" and "F" and from somatic tissue of the embryos of "C," "C_x," and "F_x." The haploid number for both "C" and "F" males is clearly 24 (fig. 6). No deviation from this number was found in cells from approximately 12 gonads that were pre-

FIG. 6. First meiotic prophase (spermatogenesis), strain "C," N: 24, 1300 x.

pared for study. The diploid chromosome number determined from the somatic tissue is 48 for the bisexual and the two unisexual strains (figs. 7 and 8).

Interspecific Matings and Genetic Analysis.—In the absence of intraspecific marker genes suitable for tracing the movement of genetic material through the unisexual strains, hybrid matings of "C" and "F" were attempted. Both sexes of "F," including the "F_x" strain, have a spot on the dorsal fin (fig. 9). This aggregate of melanophores is basally located on the posterior, interradial membranes, for the most part between rays 4 and 6. In large specimens melanophores become more numerous and in some fish cover the entire lower third of the posterior rays; at minimum development the melanin is always sufficiently concentrated to form a spot. In "C" and "C_x" this character is lacking (fig. 10). Although large females have a few melanophores scattered along the base of the dorsal fin, they are never concentrated into a spot.

In the first series of hybrid matings between "C–C_x" and "F–F_x" a clear-fin "C" female was mated to a spot-fin "F" male (Hybrid Mating I, table 3). All of the offspring had the dorsal-fin spot; but the males outnumbered the females by about 3 to 1. When the same cross was made using the unisexual female, "C_x," instead of the bisexual type (Hybrid Mating II), the fin spot was again transmitted to all of the progeny; but in keeping with the characteristic of the "C_x" strain, all of the offspring were females. The cross, "F_x" × "C" (Hybrid Mating III), as expected, also produced only females, but all of these had clear fins. Hence, in the first three crosses only the phenotype of the male was transmitted to the F_1 generation. When, however, the F_1 hybrid male ("C" × "F") which had the dorsal-fin spot was mated to "C_x" "F_x," or the F_1 ("F_x" × "C") female, half of the offspring were spot-fin and half were clear-fin (again, no males appeared).

At this point, transmission of the dorsal-fin spot would appear to be best explained by a system of sex-linkage. Although the existence of sex chromosomes has not been cytologically verified in cyprinodont fishes, the few species for which their presence has been genetically established females, with one exception, were indicated to be homogametic, XX; the males, heterogametic, XY.

When a sex-linked system with homogametic XX females was applied to the spot-fin transmission data, a fit could not be obtained, regardless of whether the spot character was considered dominant or recessive. If the female, however, were het-

FIG. 7. Mitotic metaphase, strain "C," 2 N: 48, 1300 x.

FIG. 8. Mitotic metaphase, strain "C_x," 2 N: 48, 1300 x.

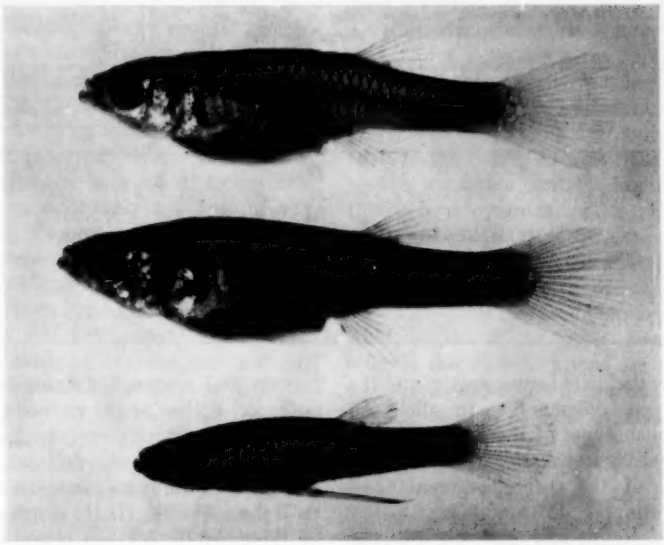

FIG. 9. Strain "F" female (upper, 27.0 mm), "F_x" female (middle, 30.2 mm), and "F" male (lower, 22.1 mm), showing dorsal-fin spot characteristic of this group.

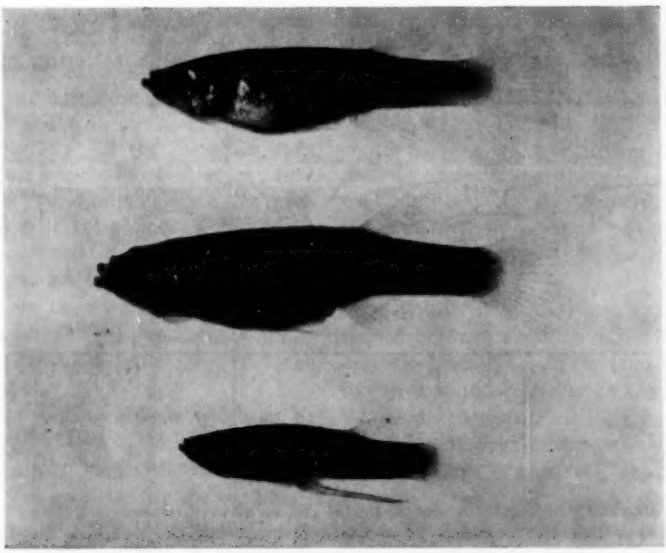

FIG. 10. Strain "C" female (upper, 22.5 mm), "C_x" female (middle, 25.1 mm), and "C" male (lower, 17.1 mm), showing clear condition of dorsal fin of this group.

TABLE 3. *First series of hybrid matings between "C–C_x" and "F–F_x," showing transmission of the dorsal-fin spot*

Hybrid mating	Female	Male	Phenotype of offspring			
			Dorsal-fin spot		No dorsal-fin spot	
			Female	Male	Female	Male
I	C	F	21	57	0	0
II	C_x	F	23	0	0	0
III	F_x	C	0	0	39	0
IV	C_x	(C × F)	39	0	46	0
V	F_x	(C × F)	4	0	2	0
VI	(F_x × C)	(C × F)	12	0	12	0
VII	(F_x × C) (C × F)	C	0	0	23	0
VIII	(F_x × C) (C × F)	C	0	0	10	0

erogametic, the male homogametic, and the dorsal-fin spot dominant to its allele, no spot, the phenotypes of the offspring from all six of the hybrid matings fulfill the requirements of the theoretical genotypes. Hybrid Matings I, III, and VI (fig. 11) show the genotype and phenotype of the parents and progeny in terms of this hypothesis. Following the method of Gordon (1952), the sex chromosomes of the heterogametic female are designated "WY" and those of the homogametic male "YY." In Hybrid Mating III, in which a spot-fin "F_x" female, W^-Y^s, was mated to a clear-

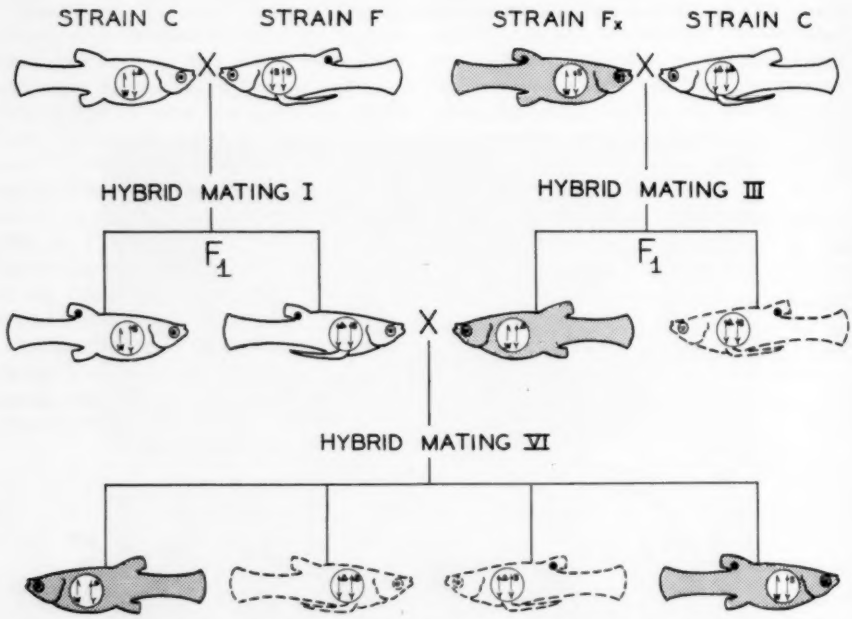

FIG. 11. Inheritance of dorsal-fin spot in hybrid crosses between strains "C–C_x" and "F–F_x," assuming females to be heterogametic (WY) and males homogametic (YY) and the spot-fin character to be dominant (S). Unisexual strains are indicated by stippling; males by presence of gonopodium. Had males been born to unisexual females, their genotypes and phenotypes would have been as represented in broken-line figures.

fin "C" male, Y^sY^s, all offspring were clear-fin females. Under normal circumstances, males should be heterozygous Y^SY^s and have the fin spot. Since males are not produced by unisexual females, however, no spot-fin progeny occurred—leaving only the W^-Y^s genotype. When these W^-Y^s F_1 hybrid females were mated to the Y^SY^s heterozygous male (Hybrid Mating VI), half of the progeny were spot-fin females and half were clear-fin females and hence were W^-Y^S and W^-Y^s, respectively. If males had been produced here their genotypes, Y^SY^s and Y^sY^s, would have resulted in half of them having the spot and half having no spot. The two types of F_2 females produced by this cross are the same genotype as "C_x" (W^-Y^s) and "F_x" (W^-Y^S). It, therefore, follows that mating these females to "C" males (Y^sY^s) should result in the same type of offspring as would be obtained by mating "C_x" or "F_x" females to "C" males, that is, all clear-fin females. The progeny from both of these crosses ($W^-Y^S \times Y^sY^s$ and $W^-Y^s \times Y^sY^s$), in Hybrid Matings VII and VIII, were, as predicted, all clear-fin females (table 3).

In the "F_x" × "C" cross, had a bisexual "F" female been used instead of the unisexual strain, progeny of both sexes would have resulted. The F_1 males produced, unlike their clear-fin, W^-Y^s sisters, would be heterozygous Y^SY^s for the dorsal-fin spot. It was not until 1959, however, after a new stock of "F" was available, that this cross was successfully completed. The 13 offspring that resulted consisted of eight females and two males, but instead of all F_1 females being clear-fin (W^-Y^s) and all males spot-fin (Y^SY^s), as the sex-linked hypothesis demands, both sexes had the dorsal-fin spot.

In Hybrid Mating XII, in which an F_1 hybrid female ("C" × "F") was mated to the clear-fin "C" male, the W^-Y^s (clear ♀), genotypes predicted by the sex-linked hypothesis again were not obtained. Instead of the females being clear-fin and the males spot-fin, a 1:1:1:1 ratio was obtained, suggesting, rather than sex-linkage, a simple Mendelian type of inheritance. In this hybrid experiment the spot character in most of the offspring was less developed than in the original "F" parent. Although individuals of the "F–F_x" group vary somewhat in the degree to which the dorsal-fin spot is expressed, critical examination of the F_1 progeny from "C" × "F" and the reciprocal cross left little doubt that the character is actually intermediate when hybrid matings involve bisexual females. This type of inheritance is in keeping with what is known of other fish hybrids, wherein the F_1 generations are almost exclusively intermediate between the parental species. In most of these hybrids, however, expression of species characters is controlled or influenced by multiple factors, such that in backcrossing to the original parents introgression is a gradual process. Expression of the dorsal-fin spot, however, does not depend on the cumulative effect of a number of genes. Apparently only one allele is involved and the heterozygous condition results in incomplete dominance, such that for either sex: (1) SS = strong-spot, (2) Ss = weak-spot, and (3) ss = no spot. In the backcrosses, then, "C" × ("C" × "F") becomes ss × Ss, which produced both male and female offspring, half of each with clear-fins, ss, and half with a weak-spot, Ss; ("C" × "F") × "C" becomes Ss × ss which again results in half clear-fin and half spot-fin progeny, whereas ("C" × "F") × "F" becomes Ss × SS, from which only spot-fin young are derived, but of two types: Ss, weak-spot, and SS, strong-spot.

"C" and "F" males and females, consequently, have chromosomes with homologous loci, which act on the same character, but with opposite effect. In the unisexual strains the corresponding locus is not found on the chromosome designated as "W" in fig. 11. The s-gene carried by either gamete of the "F" male is therefore allowed full expression in combination with the W-chromosome. Fertilization by either gamete of the "C" male, however, in com-

TABLE 4. *Second series of hybrid matings between "C–C_x" and "F–F_x" showing transmission of fin-spot character*

Hybrid mating	Female	Male	Phenotype of offspring			
			Dorsal-fin spot		No dorsal-fin spot	
			Female	Male	Female	Male
IX	F	C	11	3	0	0
X	C	(C × F)	23	20	25	35
XI	(C × F)	F	4	23	0	0
XII	(C × F)	C	4	3	6	4
XIII	(F × C)	C	8	1	3	3
XIV	(C × F)	(C × F)	12	13	1	5

bination with the W-chromosome, results in only the clear-fin condition. If males were produced from "F_x" × "C," the spot would then be present only in an intermediate form, since the F_1 male would contain an S-gene from the "F_x" female and an s-allele from the "C" male. In Hybrid Matings IV, V, and VI, wherein "C_x," "F_x," and F_1 hybrid ("F_x" × "C") females were mated to the F_1 hybrid male ("C" × "F"), the two types of male gametes, one marked by the S-gene and one by the s-allele, combined with the female gamete containing the W-chromosome to produce half spot-fin and half clear-fin daughters. If expression of the spot-fin character were controlled by multiple factors involving even as few as two loci, four types of gametes would be produced by the F_1 hybrid male: AB, Ab, aB, and ab. The first three of these combined with the W-chromosome would result in spot-fin females, whereas only the latter would produce clear-fin individuals. Since the ratio of spot-fin to clear-fin progeny in all three crosses was 1:1 and not 3:1, it is safe to conclude that multiple factors are not involved.

The phenotypes of only one of the 14 crosses (tables 3 and 4) do not conform to the expected ratio. When the F_1 offspring of "C" × "F" were inbred in Hybrid Mating XIV, a 3:1 ratio was expected. All but six of the 31 offspring, however, had either a weak or a well-developed spot. The phenotypic ratio in this cross evidently was altered by the deleterious effects of hybridization. Brood size data and embryological data indicated that 50 to 80 per cent of the would-be F_2 progeny were stillborn.

A prominent feature in several of the hybrid matings involving females of the bisexual strains is the discrepancy from a 1:1 sex-ratio. This is particularly noticeable in the "C" × "F" and ("C" × "F") × "F" matings, in which 1:3 and 1:5 ratios favored the males. In others, such as "F" × "C" and ("F" × "C") × "C," the ratio is apparently reversed, with two or three times as many females as males. Differential mortality is probably negligible, since broods sometimes contained as many as 10 young.

One female of Hybrid Mating I ("C" × "F"), that produced 39 female and 16 male offspring (all of which had the dorsal-fin spot), was remated to a male of her own species after the "F" sperm was exhausted. In the new combination she produced 47 females and 56 males (all of which lacked the dorsal-fin spot), thus restoring the 1:1 sex-ratio. In view of this result and the evidence against a lethal mechanism, it seems likely that the sex-determining factors of "C" and "F" have a difference in potential. This matter is further pursued in the discussion of sex-determination in both the unisexual and bisexual forms.

DISCUSSION

Sufficient biological and experimental evidence is now available to permit an evaluation of the *Poeciliopsis* problem in terms of what is known of one-sided sex-inheritance in other animals. Since the literature

treating this subject is too extensive for a complete review, only a few examples of the various reproductive processes are presented.

Parthenogenesis and Gynogenesis

Parthenogenesis is a common phenomenon in the animal kingdom; one form or another has been found in nearly all of the major phyla. Beatty (1957), White (1954), and Suomalainen (1950) have discussed at length the various types of parthenogenesis and have indicated that nowhere among the vertebrates has a species or strain been found that normally reproduces without fertilization. Although numerous examples may be cited in which cell division was initiated in fish, amphibian, and mammalian eggs by physical or chemical stimulation, most of the artificially produced individuals failed as early embryos. Only the Beltsville Small White turkey has produced viable progeny from unfertilized and apparently unstimulated eggs (Olsen and Marsden, 1954 and Kosin, Sato, and Nagra, 1958). All of these parthenogenetic birds were males, some of which reached maturity and yielded small amounts of semen containing motile spermatozoa (Poole, 1959).

Among the teleost fishes a few special cases are known. These involved virgin births from two females of *Lebistes reticulatus* and from one female of *Xiphophorus helleri* (Stolk, 1958). The ovaries of each female were found to be infested with the phycomycete *Ichthyophorus hoferi* Plehn-Mulsow, which apparently provided a toxic substance responsible for the artificial stimulation of the ova. More than 64 offspring were produced by this pathological parthenogenetic process; these, without exception, were females.

The famous *Mollienesia formosa,* mentioned early in this paper, was studied for 12 years by Hubbs and Hubbs (1932, 1946) and Hubbs (1955) during which time 21 generations of over 8,000 offspring were produced, all of which were phenocopies of the female parent. Isolated *M. formosa* virgins produced no young; but when placed with males of *M. latipinna* or *M. sphenops* (species with which *M. formosa* is sympatric) large broods resulted. It was concluded that *Mollienesia formosa* reproduces by a process of gynogenesis, wherein sperm is essential to initiate development of the embryo but does not actually take part in formation of the zygote.

A similar situation has since been reported (Lieder, 1955) wherein certain populations of goldfish (*Carassius auratus*) specified as the silver goldfish (*C. a. gibelio*) produced only female progeny. Here again stimulus to the ova was provided by other species, in this case a goldfish and a carp.

The evidence that parthenogenetic or gynogenetic reproduction occurs in fishes, and that three of the four species for which it has been postulated belong to the same family as *Poeciliopsis,* suggests that a similar mode of reproduction might be functional in this genus. In order for either process to provide a suitable explanation for the production of only female offspring it is essential that no paternal characters appear in any of the progeny and that the sperm serve no purpose beyond that of a stimulating agent. Although intraspecific marker genes, suitable for testing such a hypothesis are not known for "C" and "F," interspecific matings involving the transmission of the dorsal-fin spot have provided the necessary character. In Hybrid Mating II (table 3) it was demonstrated that a clear-fin "C_x" female mated to a spot-fin "F" male resulted in 23 female progeny, all of which had the spot. Crossing a spot-fin "F_x" female in Hybrid Mating III with a clear-fin male gave 37 offspring, all with the pigment condition of the male. The consistent expression of paternal characters by unisexual progeny in these and other hybrid experiments (table 3) is conclusive evidence that parthenogenesis or gynogenesis cannot explain the all-femaleness of *Poeciliopsis* strains "C_x" and "F_x."

Lethal Mechanism

Abnormal sex-ratios resulting from differential viability have been reported numerous times. Most of these, however, do not result in complete annihilation of one sex. The hybrid cross of *Drosophila melanogaster* × *D. similans* is exceptional and more closely resembles the situation in *Poeciliopsis*, in that only females are present among the mature offspring. Sturtevant (1920) found that most of the dead and dying larvae of the cross are males. A similar mechanism is known in *Drosophila prosaltens* (Cavalcanti, Falcão, and Castro, 1957). In this species, as in the two species of *Poeciliopsis*, both unisexual and bisexual strains of females are known. Although normal *D. prosaltens* mothers, like those of *Poeciliopsis* strains "C" and "F," produced no daughters of the unisexual types, the unisexual flies differed from "C_x" and "F_x" by occasionally giving rise to the bisexual form. It was believed by the above authors that the unisexual type females carry a cytoplasmic factor O (omikron), which is reproduced only in those individuals homozygous or heterozygous for the "sex-ratio" gene, S_r. The omikron plasmagene, which is transmitted exclusively through the eggs of "sex-ratio" females, kills only the XY embryos.

It is not inconceivable that a similar lethal system is responsible in *Poeciliopsis* for the lack of male offspring from strains "C_x" and "F_x." Transmission of the character of the dorsal-fin spot is not contradictory to such a mechanism. In Hybrid Mating III (fig. 11), in which "F_x" was mated to "C," the Y^sY^s genotype of the males was not represented among the F_1 progeny; only clear-fin, W^-Y^s, females were produced. In the same cross but with normal females, the Y^sY^s genotype was expressed by the F_1 males as a dorsal-fin spot of intermediate intensity.

In spite of the attractiveness of a lethal-mechanism explanation of unisexuality in *Poeciliopsis*, biological evidence offers no support. A lethal gene in poeciliids might produce its effect during either a prenatal or postnatal stage, at either of which it would be readily detectable. The percentages of deaths among offspring from the four strains of females were as follows: "C_x," 7.7%; "C," 14.2%; "F_x," 15.0%; and "F," 35.4%. From "C_x" females, entire broods of 20 and 34 offspring were raised to adulthood without a single loss. Since so few deaths occur among the progeny of the unisexual strains, a lethal mechanism is unsubstantiated for the period between birth and sexual differentiation.

It is still possible, however, for the expression of a lethal gene to be limited to the prenatal stage. One would expect under such circumstances to find brood sizes of unisexual females reduced to about half those of the bisexual strains, but no such reduction was found. The brood size of each unisexual strain was about equal to that of its bisexual counterpart (figs. 2 and 3). "C" females in the smaller size ranges produced slightly more young than "C_x" females, but larger "C_x" females had broods that were twice the size of those of any other species of *Poeciliopsis*. Furthermore, dissected ovaries of "C_x" females contained rarely more than one degenerating embryo, even when as many as 30 healthy ones were developing. The hypothesis that unisexuality is a result of a lethal system acting during either the prenatal or postnatal period must, therefore, be rejected.

Sex-Inversion

Sex-inversions have been found in a variety of animals, but the incidence is strongest in those with sex-determining mechanisms still in a primitive state (White, 1954). Cyprinodont fishes fall into this category. Their sex-chromosomes are so little differentiated as to be morphologically indistinguishable from autosomes. The sex of animals of this type is often unstable, and subject to alteration by both internal and external factors.

Among the cyprinodont fishes examples of sex-inversion are known. For example, in the guppy, *Lebistes reticulatus*, and the medaka, *Oryzias latipes*, the normal XX

(♀) – XY (♂) relationship is occasionally upset by an imbalance of minor sex-determining genes on the autosomes (Winge and Ditlevsen, 1947; Aida, 1936). Matings of these reversed XY females and XX males to normal individuals resulted in progeny with extremely abnormal sex-ratios. In the swordtail, *Xiphophorus helleri,* unusual sex-ratios have been attributed to sex-determining systems relying on hormonal control (Essenberg, 1923 and Friez, 1933).

Kosswig (1954) presented a generalized theory of sex-determination for fishes in which he stressed both environmental and genetic factors.

The evidence that sex-inversions do occur in cyprinodonts suggests that one such system might be functioning in *Poeciliopsis* strains "C_x" and "F_x." External, internal, genetic, and cytoplasmic influences have all been considered.

In rearing viviparous fishes, standardization of the external environment is impractical; consequently, offspring from each of the four strains of females were reared under all available aquarium conditions. Regardless of the light, temperature, time of year, space, food, or any other physical variable in the laboratory, females of the bisexual strains produced both male and female progeny and females of the unisexual strains produced only female young.

If sex-reversal is to be considered a characteristic of "C_x" and "F_x," it is more likely to be operative through an internal process. Histological evidence, presented above, is strongly contradictory to the concept of an inversion brought about by endocrine imbalance. Sectioned gonads from "C_x" young killed at the time of sexual differentiation revealed no evidence of male origin. This, however, does not exclude some autosomal or cytoplasmic control that may gain prominence over the sex-chromosomes prior to the formation of a distinct gonad. It is in the transmission data of the dorsal-fin spot that evidence contradicts sex-inversion in any form.

In Hybrid Mating III (fig. 2), a spot-fin "F_x" female was mated to a clear-fin "C" male. Since the gene for spot-fin is located on only one "F_x" chromosome, two types of gametes should normally occur. One of these, which is marked by a chromosome lacking the spot-gene locus, when combined with either of the clear-fin male gametes results in clear-fin females. The other female gamete, marked by the spot-fin gene, should in combination with the clear-fin male gametes result in male offspring in which the spot-fin is expressed with reduced intensity. If these spot-fin males were genetically produced but reversed to females, half of the offspring of the "F_x" × "C" cross would have a spot on the dorsal fin. It can, therefore, be concluded that autosomal or cytoplasmic factors or hormonal or physical influences do not reverse the sex of "C_x" or "F_x" males to females.

Incompatibility Mechanisms

In a number of plants, for example *Zea, Datura,* and *Oenothera,* incompatibility systems that have arisen between the style and pollen grains cause differential pollen-tube growth. Since one pollen tube may grow slower than the other, incompletely, or not at all, only one type of gamete may reach the ovary.

In viviparous fishes a similar relationship might exist between the gonaduct and the spermatozoa, whereby viability or motility of the sperm is adversely affected. If the male gametes are of two types, one carrying the female-determining factors and the other male determiners, failure of the latter to reach the ova could result in only female progeny.

Hybrid matings between "C–C_x" and "F–F_x," however, demonstrate the unlikelihood of such a system operating within the unisexual strains (table 3). Gametes of the "C" × "F" F_1 male were marked by either the clear-fin gene, s, or the spot-fin gene, S. When, in Hybrid Mating IV, a clear-fin "C_x" female was mated to this male, both phenotypes were represented

among the female progeny in a 1:1 ratio, thus demonstrating that each gamete was equally effective in fertilizing ova.

Polyploidy

Although polyploidy has been a significant factor in the evolution of plants, it has played a minor role in the animal kingdom. The fact that most animals with reduplicated sets of chromosomes or increases of one or more elements are either parthenogenetic or hermaphroditic strongly suggests that bisexual reproduction acts as a barrier to polyploidy (Muller, 1925). The claim of Svärdson (1945) and Kupka (1948) that polyploidy has occurred in salmonid fishes is based on conflicting evidence and is not widely accepted. Among the vertebrates a single triploid newt, *Triturus vulgaris*, is the only known naturally occurring example (Böök, 1940). Experimentally produced triploid and tetraploid Amphibia are not uncommon, but they invariably are sterile; consequently, it is quite unlikely that polyploidy has played a part in the evolution of Amphibia (White, 1954).

In fishes, although an exclusively female F_1 generation might be produced by an increased dosage of female determiners through polyploidy, the problem of sterility remains. Were this not so, there would still be no reason to believe that the second generation would also be all-female since segregation should result in some gametes with the normal haploid chromosome number. Inasmuch as the unisexual strains of *Poeciliopsis* have failed to give rise to a single male in five generations, it seems unlikely that polyploidy or aneuploidy is involved. Furthermore, the cytological evidence is completely negative. The haploid chromosome number of "C" and "F" males, which serve as mates to both the unisexual and the bisexual strains, is invariably 24. Embryos obtained from "C_x" and "F_x" females have the diploid number 48, which is the same as that of embryos from the bisexual strain, "C." Polyploidy can, therefore, be excluded from the list of mechanisms that might be responsible for the unisexual strains of *Poeciliopsis*.

Conclusions

Results obtained from studying the reproductive biology of *Poeciliopsis* strains "C_x" and "F_x" reduce considerably the number of possible explanations for their unisexuality. Inheritance of the dorsal-fin spot contributed most to the final conclusion. In hybrid crosses between the bisexual types, "C" and "F," the spot-fin condition is determined by a pair of alleles such that in "F," SS resulted in the fin-spot; in "C," ss resulted in no spot; and in the F_1 hybrid progeny, Ss showed incomplete dominance with the spot character present in dilute form. When hybrid matings involved either of the unisexual strains, the pattern of inheritance differed. Invariably the fin condition of the male was transmitted unaltered to the F_1 progeny. If a hybrid male (Ss) was the father, two phenotypes were represented, strong spot and no spot in a 1:1 ratio, with the intermediate condition absent.

It was concluded from a series of hybrid matings using all four strains with "C" and "F" males that the unisexual strains differ from the males and females of the bisexual strains by at least one chromosome. This nonhomologous chromosome, which for convenience was labelled "W," has no effect on the dorsal-fin spot. In the unisexual progeny expression of fin pigment depends on the paternal chromosome, "Y," which carries the gene-S for fin-spot or its allele-s for no spot. Either of these genes combined with the "W" chromosome is allowed full expression, but when paired with each other in the bisexual strains the opposing effect results in dilution.

When an "F_x" ($W{-}Y^S$) female is crossed with a "C" (Y^sY^s) male, clear-fin, $W{-}Y^s$, hybrid females are produced, but the Y^SY^s weak-spot individuals are not represented among the progeny as either males or females. The same phenomenon occurs in the "C_x" ($W{-}Y^s$) × "F" (Y^SY^S) cross. Only $W{-}Y^S$, strong-spot, females are obtained

and the Y^sY^s genotype is again lost. In these and other crosses it was noted that, regardless of whether the gametes provided by the male carried the S-gene or its s-allele, transmission occurred, yet in the unisexual females, only the W-type gamete was transmitted. If the fate of the paternal chromosome can be determined, unisexuality in "C_x" and "F_x" will be explained.

The possibility that all YY males produced by unisexual females die prior to maturity was rejected on the basis of the low prenatal and postnatal mortality; hence loss of the Y-chromosome takes place before fertilization. An almost complete lack of degenerating ova within "C_x" and "F_x" ovaries, excluded the possibility that the Y-bearing gametes died. It must, therefore, be concluded that only the W-type egg is produced and that the Y-chromosome is lost during oogenesis. In each generation, then, only the W-bearing ovum combines with the Y-bearing sperm to produce a WY female.

Although transmission data from the hybrid crosses with the unisexual females indicates a WY-chromosome system, this designation is unjustifiable in terms of the bisexual hybridization data, in which the marked chromosomes segregated randomly and behaved as autosomes. In Hybrid Mating XII, when an F_1 hybrid ("C" × "F") female was backcrossed to the clear-fin "C" male, all of the female progeny should have been W^sY^s and, hence, clear-fin, whereas all of the males, Y^sY^s, should have had the dilute spot (table 4). Since both spot-fin and clear-fin males and spot-fin and clear-fin females were produced in a 1:1:1:1 ratio, it is questionable whether the marked chromosomes are by themselves responsible for sex-determination. Consequently, there is no reason to consider the chromosomes with the spot-fin locus as sex-chromosomes. In fact there is evidence that sex-determination in "C" and "F" is in part, if not completely, dependent on genes dispersed among the autosomes. It is shown above that progeny from certain hybrid crosses have unbalanced sex-ratios, and it is suggested above that the sex-determining potential differs among the various species of *Poeciliopsis*. In the group with which we are here concerned sex-determining factors in "F" males and females are stronger than those of "C." Although these sex-determiners are balanced in intraspecific matings, the balance is not maintained in hybrid crosses. Consequently, when "C" females are mated to "F" males the stronger male-producing genes of "F" combine with the weaker female-producing genes of "C" to result in a sex-ratio of 1♀:3♂♂ (table 3). Excessive mortality was not a feature of this experiment. In the reciprocal cross ("F" × "C") stronger female-determining factors combined with the weaker male determiners, and the ratio was shifted in the opposite direction. Maturation division within the F_1 hybrids results in a mixing of the strong and weak elements, promoting an intermediate condition. The gametes of the "C" × "F" hybrid males, therefore, are not as strongly male-determining as those of "F," yet their potential exceeds that of "C." When such a male is backcrossed to a "C" female, male offspring still slightly outnumber the females, but not significantly so, since differential mortality tends to equalize the ratio. In the "C" × "F" hybrid female, the same mixing of sex-determiners takes place, to result in ova that tend toward maleness. Combining these gametes with strong male-determining gametes of "F" results in nearly all male progeny: only 4 out of 27 were females (table 4).

Hybrid crosses involving members of the genus *Poeciliopsis*, not considered in this study, suggest that a similar situation exists for other closely related species. In some the difference in potential of sex factors is greater than in "C" × "F," whereas in others it is less defined.

This relationship of strong and weak sex-determiners bears some resemblance to the genetic mechanism in the gypsy moth, *Lymantria dispar*. In this species the male- and female-determining factors are in equilibrium in pure-bred races, but in in-

terracial crosses involving "weak" females and "strong" males the F_1 generation is completely male in appearance (Goldschmidt, 1931, 1932, 1934). Half of these, however, proved to be intersexes. In *Lymantria*, females are heterogametic, so that the X- (or Z-) chromosome is male-determining and variable in potency, but the female-determining factors evidently are cytoplasmic.

Although interracial crosses of *Lymantria dispar* and interspecific crosses of *Poeciliopsis* are comparable on the basis of varying degrees of sex-determining potential, here the similarity ends. In *Lymantria* the ratio of males to females is not really shifted but intersexes take the place of what normally would constitute one of the sexes. Furthermore, in F_1 hybrids of *Poeciliopsis* the sex-determining factors are redistributed in such a way that equilibrium is eventually restored, as would be true with polygenic inheritance.

If in the bisexual strains, sex is determined totally or in part by a number of genes dispersed among the autosomes, the hypothesis that femaleness in "C_x" and "F_x" is attributable to a single chromosome referred to as "W" is open to question. It is a more reasonable assumption that sex here is also controlled by a number of genes. Since chromosomes carrying these genes do not undergo random assortment during oogenesis, but behave as a unit, interpretation of the data is the same as though a single nonhomologous sex-chromosome were involved. Genes carried by this unit are responsible for such differences between the bisexual and unisexual strains as: dentition, ova size, size of young at birth, maximum size of adults, age and size at maturity, etc.

It is probable that in the recent past a species of *Poeciliopsis*, not closely related to "C" or "F," through hybridization altered the genomes of the strains now known as "C_x" and "F_x." Some of the foreign chromosomes were retained by the unisexual strains in subsequent backcrosses. These chromosomes not only carry genes responsible for the modified characters noted above but also have female-determining factors too strong to be counterbalanced by the male determiners on the corresponding chromosomes. In hybrid combinations between closely related species of *Poeciliopsis*, such as "C" and "F," the high degree of genic compatibility permits normal pairing and segregation of chromosomes during meiosis. The "new" chromosomes contained by "C_x" and "F_x," however, are too dissimilar to pair with the male chromosomes. Consequently, paternal characters expressed by each generation fail to be incorporated into the F_1 ova and the two unisexual strains remain perpetual hybrids.

In vertebrate animals selective maturation has not been previously verified either genetically or cytologically, but details of the process have been worked out for a number of insects in the families Margaroidae, Lacanoidae, Sciaridae, Cecidomyidae, and Pediculidae (White, 1954). The means by which chromatin is eliminated varies. In general, oogenesis is normal but in the spermatogonial divisions certain chromosomes or the entire genome of one sex or the other is discarded in a bud of cytoplasm resembling a polar body. Expulsion of certain chromosomes may even occur during early cleavage stages.

With the information now at hand the two examples of unisexuality in the family Poeciliidae can be compared. Basically the two mechanisms are different. In *Mollienesia formosa*, fertilization does not include gametic fusion; hence the phenotype of the offspring is totally dependent on the genotype of the mother; the all-female progeny of *Poeciliopsis*, however, express characters of both parents. In spite of this basic difference there are ways in which the two systems are alike. In *M. formosa* the egg nucleus contains only maternal chromatin; the same is true in mature ova of *Poeciliopsis* strains "C_x" and "F_x" since the paternal chromatin (for the most part at least, if not in total) is lost during meiosis. In individuals with either type of

mechanism, the unisexual condition prevents mutations from becoming part of a common gene pool. Any change is restricted to the clone in which it originates. The obligatory heterozygous nature of the unisexual *Poeciliopsis,* however, would seem to hold certain advantages over the gynogenetic type of *Mollienesia* in that each generation of "C_x" or "F_x" utilizes the genetic variability originating within the bisexual strains. Since these paternal contributions are not incorporated in the "C_x" and "F_x" genetic systems but are merely "on loan," the unisexual strains are, in effect, as isolated from their bisexual counterparts as is *Mollienesia formosa* from its males of *M. latipinna* and *M. sphenops*. It is possible that the selective segregation of *Poeciliopsis* represents a transition stage to gynogenesis and thence to parthenogenesis, but it is equally possible that these two unisexual mechanisms are merely leftovers from the hybridization of animals with inefficient isolating mechanisms.

If the latter situation obtains, it is difficult to imagine that such a dead-end phenomenon has persisted or could long persist in nature. It, therefore, might still be possible to reconstruct the unisexual strains. Investigations currently being conducted toward this end have been promising; and although a unisexual type has not been produced, male progeny were obtained when "C_x" was mated to males of a different species group. It is believed that with continued experimentation in this area the "C_x" and "F_x" strains will ultimately be synthesized in the laboratory.

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Summary

1. The viviparous poeciliid fishes of the genus *Poeciliopsis* comprise approximately 16 species that chiefly inhabit the Pacific slope of Mexico but occur as far south as Colombia. Two allopatric populations, here designated "C" and "F," each have, in addition to normal males and females, an aberrant, unisexual strain. These strains, "C_x" and "F_x," in five laboratory generations, gave birth only to female progeny. In attempting to determine the mechanism by which all-female broods are produced, the reproductive biology and genetics of both "C–C_x" and "F–F_x" were investigated.

2. In *Poeciliopsis* fertilization is internal. The embryos develop within the ovarian follicles by a process of superfetation, whereby two or more stages of embryos occur in the ovary simultaneously. Births occur at intervals of 7 to 14 days, with as many as 34 offspring in a single brood; however, broods of fewer than 12 are more common.

3. Examination of ovaries and brood data collected over the four-year period of this investigation eliminated the possibility that the lack of male offspring from "C_x" and "F_x" females is caused by a lethal system operative on either the gametes, embryos, or juveniles. Genetic data and examination of sectioned gonads provided evidence against sex-inversion. Inheritance of paternal characters by the unisexual type progeny invalidates parthenogenesis or gynogenesis, and polyploidy was rejected on the basis of chromosome counts.

4. In hybrid crosses between "C" and "F" there is a high degree of genetic compatibility. Meiotic divisions of the F_1 progeny are apparently normal, with random segregation of chromosomes. Reciprocal differences in the sex-ratio suggest, however, that the potency of the sex-determining factors of one species exceeds that of the other. There is no genetic or cytological evidence that the bisexual strains have sex chromosomes. It appears that, to a large extent if not completely, sex-determination depends on autosomal genes.

5. Inheritance of a marker gene (dorsal-fin spot) in both the bisexual and the unisexual strains bears significantly on the mechanism involved in the production of all-female broods. When crosses involved the bisexual-type female, transmission data indicated autosomal inheritance with incomplete dominance. When the unisexual strains were used in the same crosses, however, the character appeared to be sex-linked with the female heterogametic, WY, and the fin-spot (S) dominant to its allele, no-spot (s). Expression of the character in each "all-female" generation was, therefore, totally dependent upon the S locus of the paternal chromosome. All evidence indicated that the male chromosome is not transmitted through the F_1 ova but is lost during oogenesis.

6. It is postulated that the nonhomologous or "W" chromosome of "C_x" and "F_x" is in reality not a sex-chromosome, but rather one of several chromosomes introduced into the genomes of the unisexual females through hybridization. These chromosomes, which are inherited as a unit, have female-determining factors too strong to be counterbalanced by the paternal factors provided by "C" or "F" males. They furthermore are genetically too dissimilar to pair with the paternal chromosomes during oogenesis. Asynapsis probably results in the expulsion of the paternal chromosomes in a polar body.

7. The only other naturally occurring unisexual vertebrate is *Mollienesia formosa*, a cyprinodont fish in the same family as *Poeciliopsis*. The reproductive mechanisms of these two exclusively female-producing groups, however, are different. In *M. formosa* sperm provided by males of two other species serves only to stimulate the ova into development, there being no gametic fusion, whereas in *Poeciliopsis* fertilization is normal and paternal characters are expressed by the offspring.

Literature Cited

Aida, T. 1936. Sex reversal in *Aplocheilus latipes* and a new explanation of sex differentiation. Genetics, **21**: 136–153.

Böök, J. A. 1940. Triploidy in *Triton taeniatus* Laur. Heridatas, **26**: 107–114.

Cavalcanti, A. G. L., D. N. Falcão, and L. E. Castro. 1957. "Sex-ratio" in *Drosophila prosaltens*—a character due to interaction between nuclear genes and cytoplasmic factors. Amer. Nat., **90**: 327–329.

Essenberg, J. M. 1923. Sex-differentiation in the viviparous teleost *Xiphophorus helleri* Heckel. Biol. Bull., **45**: 46–97.

Friesz, E. 1933. Untersuchungen über die Geschlechtsumkehr bei *Xiphophorus helleri* Heckel. Roux Arch. Entw. Mech. Organ., **129**: 255–355.

Goldschmidt, R. 1931. Analysis of intersexuality in the gypsy moth. Quart. Rev. Biol., **6**: 125–142.

——. 1932. Untersuchungen zur Genetik der geogrophischen Variation. III. Abschliessendes über die Geschlechtsrassen von *Lymantria dispar*. Roux Arch. Entw. Mech. Organ., **126**: 277–324.

——. 1934. *Lymantria*. Bibliogr. Genet. **II**: 1–186.

Gordon, M. 1952. Sex determination in *Xiphophorus* (*Platypoecilus*) *maculatus*. III. Differentiation of gonads in platyfish from broods having a sex ratio of three females to one male. Zoologica, **37**: 91–100.

——. 1957. Physiological genetics of fishes. *In* Physiology of Fishes. Vol. 2. Acad. Press Inc., New York. pp. 431–501.

Hubbs, C. L. 1955. Hybridization between fish species in nature. Syst. Zool., **4**: 1–20.

—— and L. C. Hubbs. 1932. Apparent parthenogenesis in nature, in a form of fish of hybrid origin. Science, **76**: 628–630.

—— and ——. 1946. Breeding experiments with the invariable female, strictly matroclinous fish, *Mollienisia formosa*. Rec. Gen. Soc. Am., No. **14**: 48.

Kosswig, C. 1954. Zum Geschlechtsbestimungsproblem bei den Zahnkarpfen. Rev. Fac. Sci. Univ. Istanbul, **19**: 187–190.

KOSIN, I. L., I. SATO, AND H. NAGRA. 1958. Studies of parthenogenesis in the domestic turkey and chicken. Eleventh World's Poultry Congress, Mexico City, Mexico.

KRUMHOLZ, L. A. 1948. Reproduction in the western mosquitofish, *Gambusia affinis affinis* (Baird and Girard), and its use in mosquito control. Ecol. Monogr., **18**: 1–43.

KUPKA, E. 1948. Chromosomale Verschiedenheiten bei schweitzerischen Coregonen (Felchen). Rev. Suisse Zool., **55**: 285–293.

LIEDER, U. 1955. Männschenmangel und natürliche Parthenogenese bei der Silberkarausche *Carassius auratus gibelio*. Naturwissenschaften, **42**: 590.

MAKINO, S. 1951. An atlas of the chromosome numbers in animals. Iowa State College Press, pp. 1–290.

MILLER, R. R. 1960. Four new species of viviparous fishes, genus *Poeciliopsis*, from northwestern Mexico. Occ. Papers Mus. Zool. Univ. Mich., No. **619**: 1–11.

—— AND R. J. SCHULTZ. 1959. All-female strains of the teleost fishes of the genus *Poeciliopsis*. Science, **130**: 1,656–1,657.

MULLER, H. J. 1947. The production of mutations. Jour. Hered., **28**: 259–270.

OLSEN, M. W., AND S. J. MARSDEN. 1954. Natural parthenogenesis in turkey eggs. Science, **120**: 545–546.

ÖZTAN, N. 1954. Cytological investigation of the sexual differentiation in the Anatolian cyprinodontids. Rev. Fac. Sci. Univ. Istanbul, **19**: 245–280.

POOLE, H. K. 1959. The mitotic chromosomes of parthenogenetic and normal turkeys. Jour. Hered., **50**: 151–154.

STOLK, A. 1958. Pathological parthenogenesis in viviparous toothcarps. Nature, **181**: 1,660.

STURTEVANT, A. H. 1920. Genetic studies on *Drosophila simulans*. I. Introduction. Hybrids with *Drosophila melanogaster*. Genetics, **5**: 488–500.

SUOMALAINEN, E. 1950. Parthenogenesis in animals. Advanc. Genet., **3**: 193–253.

SVÄRDSON, G. 1943. Studien über den Zusammenhang zwischen Geschlechtsreife und Wachstum bei *Lebistes*. Meddel. Rept. Swedish State Inst. Freshw. Fish. Res. Drottningholm, **21**: 1–48.

——. 1945. Chromosome studies in Salmonidae. *Ibid.*, **23**: 1–151.

TURNER, C. L. 1937. Reproductive cycles and superfetation in poeciliid fishes. Biol. Bull., **72**: 145–164.

WHITE, M. J. D. 1954. Animal cytology and evolution. Cambridge Univ. Press. 454 pp.

WICKBOM, T. 1943. Cytological studies in the family Cyprinodontidae. Hereditas, **29**: 1–24.

WINGE, Ö., AND E. DITLEVSEN. 1947. Color inheritance and sex determination in *Lebistes*. Heredity, **1**: 65–83.

DIRECTIONS OF DIFFERENTIATION IN POPULATIONS OF WILD RICE, *ORYZA PERENNIS* AND *O. SATIVA* F. *SPONTANEA*[1]

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The wild rice widely spread in swampy lands of tropical Asia has been classified into *Oryza perennis* Moench. and *O. sativa* f. *spontanea* Roschev. *O. perennis* is a perennial grass with rhizomes which usually grows in deep-water swamps (Chatterjee, 1948). *O. barthii* A. Cheval. (Africa), *O. longistaminata* A. Cheval. et Roehr (Asia and Africa), and *O. cubensis* Ekman (Latin America) may belong to this species (Sampath and Rao, 1951; Chatterjee, 1948 and 1951). *O. sativa* f. *spontanea* is characterized by annual habit and is found in marshy lands or in cultivated rice fields. Its known distribution is limited to Asia. Though given the systematic rating of a form of *sativa*, it has often been regarded as a distinct species for which several synonyms have been used, such as *O. fatua* Konig, *O. rufipogon* Griffith, etc. (Chatterjee, 1948).

The authors assume that from the taxonomic point of view, the same rank may be attributed to *O. perennis* of Asia and *O. sativa* f. *spontanea*. Therefore, the two taxa will be called in this paper "*perennis*" and "*spontanea*" types of wild rice. Both, however, show wide variations in various characters, as early pointed out by Watt (1891) as well as by Roy (1921); a continuous array of intergrades was found between them.

The two types of wild rice have the same chromosome number ($n = 12$), and the basic botanical characters of the cultivated rice, *O. sativa* L. Their F_1 hybrids with cultivated varieties usually show no disturbance in chromosome pairing, and are fertile to varying degree (Sampath and Rao, 1951; Morinaga and Kuriyama, 1956; Nezu, Katayama, and Kihara, 1960). It is also known that natural hybridization occurs between them and cultivated rice (Hector, 1935; Sampath and Govindaswamy, 1958; Oka and Chang, 1959). Thus they may both be regarded as genetically in close relation with *O. sativa*, and as Roschevicz (1931) has assumed, are probably the progenitors of cultivated rice.

Considering the origin of cultivated rice, our question was how these two types of wild rice are related to each other, and which may be regarded as the ancestor. It seemed necessary to look into the pattern of variation in this group of wild forms and to compare it with variation in cultivated rice. Collecting tours were made to India, Thailand, Malaya and other countries, where seeds were collected from a number of natural populations of wild rice. Plants from the seeds were investigated in Misima, Japan as well as in Taichung, Formosa. Based on the results of these investigations, the authors deal in this paper with the discrimination between *perennis* and *spontanea* types, and genetic structure of populations and other features related to the difference in breeding habit between the two types.

MATERIALS AND METHODS

1. *Wild populations and their habitats.*—The first junior author visited the Bengal Bay coastal regions of India and Ceylon from October to December, 1957, and Thailand from November to December, 1958. Dr. T. Hirayoshi of Gifu University, Japan, also visited Malaya in January and February, 1959. They collected many seed

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samples from natural populations of *perennis* and *spontanea*, each representing a population (mixture of seeds of many plants taken at random from a population, or seeds taken on an individual plant basis from a population).

Generally, in India and Thailand the rainy season comes in May and ends between late September and early October, whereas equatorial regions such as Malaya and Ceylon have the benefit of both summer and winter monsoons. Wild rice generally carries through the vegetative growth in the rainy season and reaches maturity in the early dry season with shortening day-length in the fall. The populations called *perennis* type in this paper were mostly found in water reservoirs or in creeks along road embankments, the depth of the water being more than half a meter from November to December when the seeds were collected. The populations called *spontanea* type were found in shallow swampy patches or in mostly parched lands. Some populations of both the *perennis* and the *spontanea* types were found in paddy fields or their proximity, and only a few seemed to be completely isolated from cultivated rice.

2. *Conditions of experiments.*—Plants from the collected seeds were raised in a greenhouse or in automatic shortday fields at the National Institute of Genetics, Misima, Japan, and also in the experimental field of the Taiwan Provincial College of Agriculture, Taichung, Formosa, in 1958 and 1959. Monthly average temperatures and photoperiods at Misima (35° N), Taichung (24° N), and Bangkok (13° N, representative of the tropical countries from which our materials were collected) are shown in fig. 1, together with the temperatures in our greenhouse and the day-lengths in our shortday fields.

The dates of water soaking of seeds for germination were, in Misima, middle April for shortday field culture, and middle June for greenhouse culture. Those in Taichung were May 23 and June 22, respectively. In Misima, about one-half of the standard

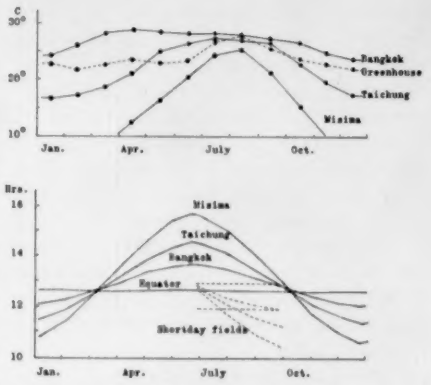

FIG. 1. Monthly average temperatures and photoperiods at Bangkok (13° N), Taichung (24° N) and Misima (35° N), as compared with those in our greenhouse and shortday fields.

mixture of fertilizers (4 N, 4 P_2O_5, and 4 K_2O in gm/m^2) was applied, but no fertilizer was used in Taichung where the soil fertility was high enough. In Misima 70 and in Taichung 29 populations were observed. The number of plants per population was 40 to 100 in Taichung, but in Misima, owing to limited space, only three to five plants per population could be raised.

In addition to the wild populations, about eighty cultivated strains from various Asian countries (formerly used for phylogenetic studies of rice varieties; Oka, 1953, etc.) were grown as controls.

3. *Characters investigated.*—Records were taken on an individual plant basis regarding the following characters in both Misima and Taichung: Spikelet length, spikelet width, apiculus hair length, awn length, anther length, ligule length, panicle length, rachis number per panicle, spikelet number per panicle, weight of 100 grains, plant height, heading date, pollen fertility, degree of grain shedding, seed dormancy, regenerating ability, resistance to potassium chlorate, resistance to low temperatures, and photoperiodic response. Some of the measuring methods are briefly described as follows:

Degree of grain shedding: Panicles were

run over by a rubber roller (500 gm, 3 cm in diameter) six times on a board inclined at 3°, and the number of grains dropped before and after this test was recorded in per cent of the total grain number.

Seed dormancy: Germination tests were made three times, 50, 90, and 180 days after heading, with hulled and unhulled seeds. The seeds to be tested were stored in desiccators with calcium chloride at room temperatures, and were tested on moistened filter paper in petri dishes at 30° C for 10 days. From each test, the percentage of germination and the mean number of days for germination were obtained for a sample. To evaluate the germinating activity in general, the following formula was used:

$$\text{Germin. activity} = (\%\ \text{of germin.}) \times \frac{11 - \text{no. of days for germin.}}{10}$$

Germinating activity gradually improved with increase in the number of days after heading. The number of days required for the above index for germinating activity to reach 40% (in hulled seeds) or 20% (in unhulled seeds) was calculated as a relative measure of dormancy.

Regenerating ability: Plants were cut at maturity about 5 cm above the roots, and the percentage of plants with new shoots was recorded about one month after cutting.

Resistances to potassium chlorate and to low temperatures: These two characters are known to distinguish the Indica from the Japonica types of *O. sativa* (Oka, 1953, 1958a). The former was shown by the concentration of $KClO_3$ solution (in logarithms) which causes a certain medium degree of damage, after treating seedlings with different concentrations. Resistance to cold was shown by index-numbers representing the degree of damage to seedlings treated at 0°–1° C for three days.

Photoperiodic response: In Taichung, plants of a photoperiodically insensitive strain, seeded in May, produce panicles one month earlier than those seeded in June; with a highly sensitive strain, plants seeded in May and in June head at the same date in fall. "Photo-sensitivity index" (in TDM degrees) and "critical daylength" were measured on the basis of this behavior by the method used by Oka (1954, 1958b). The data obtained in Misima by using shortday fields are not presented in this paper in view of the limited number of populations so far observed.

Results of Observations

1. *Inter-populational Variations in Various Characters*

Propagating habit.—The name *perennis* attributes perennial habit to the species. In fact, wild plants found in deep-water swamps, presumably representing this form, often showed rhizomes. However, populations intermediate between *perennis* and *spontanea* types were often found. Though the breeding behavior of a natural population is not easily observed, regenerating ability under experimental conditions may be regarded as an expression of asexual propagation in a state of nature. Also the presence or absence of sterility may be related to the sexual *vs.* asexual propagating habit of a population.

Among the populations investigated, the percentage of regenerating plants ranged from 0% to 100% in Misima (greenhouse) as well as in Taichung (experimental field). The data from the two places were comparable for most of the wild populations; they were then averaged for each population. The percentage of partially fertile plants (pollen fertility lower than 85%) was observed in Taichung.

Distributions of the percentages of regenerating and of partially fertile plants among the populations investigated are shown in fig. 2. The scatter diagram shows that both percentages vary in a continuous range, while populations with high regenerating ability tend to show a high frequency of partially fertile plants. This indicates that in a population with high regenerating ability, sterile plants are not necessarily eliminated on account of their asexual propagation. The figure also shows that populations with high regenerating

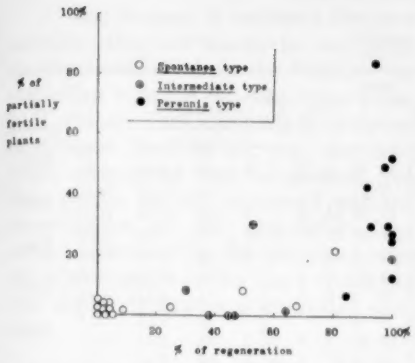

FIG. 2. Variations in regenerating ability and fertility among wild populations.

ability and low fertility belong to the *perennis* type, and those with low regenerating ability and high fertility to the *spontanea* type, if compared with the results of our classification which will be mentioned later.

Dormancy of seeds. — Pronounced dormancy is a characteristic feature of wild rice as compared with cultivated rice. Distributions of the number of days required for overcoming dormancy, found from the data obtained in Misima, are shown in table 1. As the table shows, when unhulled seeds were tested, most wild strains did not overcome dormancy within a six-month period, in contrast to cultivated varieties whose seeds germinated well 30 to 60 days after heading. Dormancy may be a useful character for wild rice as it is for many other wild plants. However, testing with hulled seeds, some strains classified as the intermediate *perennis–spontanea* type showed a relatively rapid overcoming of dormancy. It is interesting to note that among wild populations, some show a weak dormancy which resembles that of the cultivated plants.

The number of days required for overcoming dormancy may differ according to the environmental conditions during maturity as well as the conditions of seed storage. Compared with the data obtained in Misima, Taichung generally showed better germination or a more rapid overcoming of dormancy, especially for strains of the *spontanea* type.

Quantitative characters.—Wild populations differ in various morphological traits. The distributions of population means among wild populations were compared with those among cultivated varieties from various Asian countries, dealing with a number of characters. In table 2 the frequency distributions are shown by dividing the variation ranges into 11 classes, scored from 0 to 10; the original class values can be read by adding the product of class interval and score to the basic value given in the table.

The data in table 2 prove that all the characters investigated showed a continuous array of intergrades among wild populations; no tendency toward bimodality of distribution could be found. This suggests

TABLE 1. *Variation in number of days required for "germinating activity" to reach 40% (with hulled seeds) or 20% (with unhulled seeds)*[1]

Seeds tested	Type	No. of days after heading								No. of strains
		30	60	90	120	150	180	210	240 or more	
Hulled seeds	*Perennis*	2	2	3	1	3	3			14
	Intermediate	6	2		1		2	1		12
	Spontanea	3	1	3	1	3	1	1	4	17
	Cultivated	86	4		2					92
Unhulled seeds	*Perennis*	1		1	2	1	2	1	6	14
	Intermediate	1	1		3	1			6	12
	Spontanea		1				1		15	17
	Cultivated	67	22	1	2					92

[1]Data obtained in Misima.

TABLE 2. *Variation in several characters among wild populations as compared with cultivated varieties*

Character	Class interval	Group	Score										No. of populs.	Mean	S.D.	t^1
			0	1	2	3	4	5	6	7	8	9	10				
Spikelet	0.4 mm	Wild			1	3	16	23	14	13				70	8.26	0.46	0.23
length	(0 : 6.2 mm)	Cult.	1	4	7	12	13	19	15	4	1	2	2	80	8.05	0.83	
Spikelet	0.25 mm	Wild	5	6	15	29	12	3						70	2.41	0.23	1.77
width	(0 : 1.75 mm)	Cult.			1	9	14	23	14	14	3	1	1	80	3.08	0.30	
Lth/width	0.25	Wild					2	8	25	15	11	8	1	70	3.44	0.26	1.60
ratio	(0 : 1.75)	Cult.	2	6	17	13	12	14	11	3		1	1	80	2.70	0.38	
Apiculus	0.1 mm	Wild					3	34	17	2				56	0.533	0.063	0.98
hair length	(0 : 0 mm)	Cult.	3		24	19	7	8	7	3	2			73	0.345	0.181	
Weight of	0.2 gm	Wild	2	5	13	13	10	5	1					49	1.88	0.27	0.84
100 grains	(0 : 1.3 gm)	Cult.	1	2	9	9	12	19	10	8	7	5	2	84	2.32	0.45	
Awn length	1.0 cm	Wild	1	3	1	5	5	9	14	12	5	4	6	65	5.99	2.38	1.81
	(0 : 0 cm)	Cult.	68	3		2	4	6	1					84	0.73	1.65	
Anther	0.4 mm	Wild	1	6	6	10	10	5	5	1	3	3	2	52	3.50	1.02	1.11
length	(0 : 1.8 mm)	Cult.	9	18	18	2								47	2.31	0.33	
Ligule	0.4 cm	Wild	1	3	10	12	12	13	10	3				64	2.18	0.67	0.99
length	(0 : 0.6 cm)	Cult.	5	6	15	10	4	5						45	2.09	0.49	
Plant	20 cm	Wild	1	2	2	7	6	5	4					27	124	32.4	0.52
height[2]	(0 : 50 cm)	Cult.			3	9	21	30	15	6				84	145	23.9	
Panicle	2 cm	Wild		4	3	5	18	16	16	3	3			68	19.4	3.26	0.25
length	(0 : 10 cm)	Cult.	1	1	2	10	16	31	26	10	4	2	2	106	20.0	3.58	
Rachis no.	1	Wild	4	10	5	10	17	11	4	5	2			68	7.7	2.04	0.73
per panicle	(0 : 4)	Cult.	2	1	1	7	14	24	24	7	11	9	6	106	9.9	2.22	
Spikelet no.	20	Wild	8	19	23	9	4	2	1	2				68	45.7	15.3	1.54
per panicle	(0 : 20)	Cult.	1	2	4	18	28	20	15	6	8	2	2	106	110.6	39.1	
% of	10%	Wild					2	2	1	5	6	4	7	27	78.8	18.7	3.61
shedding[2]	(0 : 0%)	Cult.	43	32	7	2								84	6.2	7.4	
$KClO_3$	0.2 in log.	Wild		11	6	6	8	6	7	1				45	−3.23	0.38	0.32
resistance	(0 : −3.90)	Cult.	5	26		1	3	3	6	10	10	13	1	78	−2.98	0.69	
Low temp.	0.1	Wild						3	2	4	3	12	18	42	0.87	0.16	2.38
resistance	(0 : 0)	Cult.	24	24	10	7	8	6	2	4	2		1	88	0.23	0.22	

[1] For the difference between wild and cultivated plants. t values for $n_1 = n_2 = 1$ are given.
[2] Records taken in Taichung; other characters recorded in Misima.

that the *perennis* and *spontanea* types cannot be discriminated by any single character.

Comparing the wild and cultivated groups, marked differences are found in the percentage of shedding, spikelet number per panicle, awn length, low temperature resistance, and some other characters. When the relative magnitude of the differences was evaluated by t values, shedding gave the largest value; most wild populations shed their grains at maturity, in sharp contrast with the cultivated plants. In other characters, however, variation ranges of wild and cultivated plants overlapped.

We may then compare the ranges of variation, or the standard deviations, of population means between the wild and cultivated groups. It is found in table 2 that in anther length, ligule length, awn length, and plant height, wild populations have wider ranges of variation than the cultivated varieties. These characters might be sensitive to the conditions which differ among the habitats of wild populations. They take part in making up different character complexes of the *perennis* and *spontanea* types, as will be demonstrated later.

In contrast to the above, in spikelet length, apiculus hair length, potassium chlorate resistance, and low temperature resistance, wild populations are relatively uniform and cultivated varieties vary in a wide range. These characters might be taking part in the differentiation of cultivated

varieties; they are known to distinguish the "Continental" from the "Insular" or the Indica from the Japonica types (Oka, 1953, 1958a). Thus, according to responses to different kinds of selection, the characters investigated may be assorted into three groups, the first concerned with the diversification of wild populations, the second differentiating the cultivated from the wild types, and the third concerned with the diversification of cultivated varieties.

2. *Discrimination between* perennis *and* spontanea *Types by Multivariate Analysis*

As mentioned in the previous section, the *perennis* and the *spontanea* types cannot be clearly discriminated by a single character. It seems that they form a group in which various character associations occur. Data of population means for the following five characters were analyzed by the technique of principal component analysis, which can derive a few variation axes from a multivariate group. The characters chosen were anther length (A), rachis number per panicle (R), ligule length (L), panicle length (P), and length/width ratio of spikelets (G). Correlation coefficients among these characters were calculated for sixty wild populations from the data obtained in Misima. From the matrix of these correlation coefficients, the latent roots (λ_1 and λ_2) and corresponding vectors (l_1 and l_2) of the characteristic equation for the first and second components were computed by the method described by Kendall (1957). From the values of λ_1 and λ_2, it was found that 35% and 29% of the total multivariate variation were extracted by the first and second components, respectively. The first and second latent vectors, l_1 and l_2, gave the following component axes, defining a plane on which the populations under observation could be scattered.

$X_1 = 1.00\ A - 0.52\ R + 0.26\ L + 0.44\ P + 0.94\ G$, and

$X_2 = 0.12\ A + 0.86\ R + 1.00\ L + 0.74\ P - 0.28\ G$.

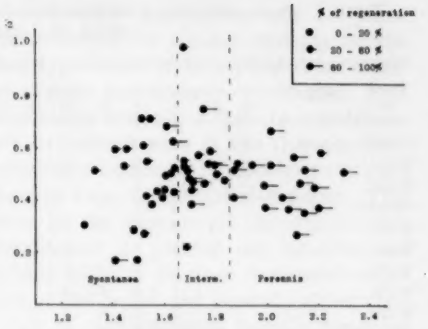

FIG. 3. Populations of *O. perennis* and *O. sativa* f. *spontanea* scattered in the plane defined by the first and second component axes.

The scatter diagram thus obtained is shown in fig. 3. The diagram shows that no particular clusters of population occur in the multivariate group, i.e., the variation among populations is continuous. This computation does not give, however, the biological meaning of the component axes. In an attempt to find their meaning, two discriminant functions were computed by the method of Fisher (1936), one (Y_1) to maximize the difference between the *perennis* and *spontanea* types which were empirically determined, and the other (Y_2) to maximize the difference between wild and cultivated plants. For constructing the formula Y_1, data for 10 *perennis* and 20 *spontanea* populations showing typical characteristics of those types were used. For the formula Y_2, data for 20 wild populations (10 *perennis* and 10 *spontanea*) and 20 cultivated strains were used. The formulas thus obtained (standardized) were

$$Y_1 = 1.00\ A - 0.02\ P + 0.46\ L + 0.64\ G,$$

and

$$Y_2 = 1.00\ S + 0.38\ D - 0.20\ W - 0.13\ N,$$

where A, P, L, and G are the same notations as used in the principal component analysis, and S, D, W, and N represent degree of grain shedding, seed dormancy, 100 grain weight and spikelet number per panicle, respectively.

TABLE 3. *Inter- and intra-population variation of the score given by a discriminant function* (from data obtained in Taichung)

Popul. no.	Type[1]	Score															Mean	S.D.
		–20	–10	0	10	20	30	40	50	60	70	80	90	100	110	120	130	
W106	*Spont.*	4	8	8														–8.0	6.3
W101	,,	1	5	11	2		1											–0.6	8.6
W107	,,		3	10	7													1.0	6.1
W204–6	,,		1	4	12	7												10.0	7.2
W203	,,		1	5	6	6	1		1									12.8	7.5
W168	,,	3	1	1	3	7	2	2	1									14.6	20.5
W105	,,			2	8	8	1	1										14.9	10.2
W201	,,			1	8	7	4											16.7	9.0
W209	,,			2	6	6	2	1	2									20.0	18.8
W170	,,			5	1	7	3	3	1									21.6	15.2
W139	,,				1	3	7	6	1	2								33.7	12.4
W173	,,		1	2			1	2			1	1						33.9	34.1
W179	,,					2	4		8	4	1		1					47.3	16.1
W164	*Interm.*						5	2	1			3						47.9	21.5
W134	,,					2	1	4	7	2	2			1				51.8	21.6
W136	,,						2	3	8	2	3	1	1					53.9	15.3
W133	,,						1	6	5	1	2	3	2					57.1	18.2
W167	,,						1		1	2	2	2	4	2	2			80.0	23.1
W120	*Peren.*							1	2	2	7	5	9	5	6	3		87.1	21.1
W171	,,								1	1	2	2	2		3	2		89.6	24.2
W208	,,							2	1		2		1	2	4	2		89.7	29.3
W202	*Interm.*										2		4	6	1	1	1	96.4	16.2
W109	*Peren.*									1		1	2	1	3		2	102.5	21.0
W132	,,											1	4	2	7	2	4	108.0	15.6

[1] Classified on the basis of component analysis.

Correlations between the scores given by X_1, X_2, Y_1, and Y_2 were as follows:

$$r_{X_1Y_1} = 0.906,\ r_{X_2Y_2} = 0.519,$$

$$r_{X_1Y_2} = -0.092,\ r_{X_2Y_1} = 0.306,\ \text{and}$$

$r_{Y_1Y_2} = -0.181$. ($r_{X_1X_2} = 0$, theoretically.)

The high correlation between X_1 and Y_1 indicates that the first component axis represents the differentiation of wild populations into *perennis* and *spontanea* types. In fig. 3, it is also found that the X_1 score is correlated with regenerating ability. According to the X_1 scores, the populations investigated were conventionally classified into *perennis, intermediate,* and *spontanea* types, as shown by broken lines in fig. 3. This classification will be used in this paper for various comparisons. The correlation between X_2 and Y_2 may be said to indicate that the wild populations vary in the direction of the cultivated type and this tendency is found as the second component axis.

A further attempt was made to investigate the pattern of variations in this group. Using data for 12 populations taken in Taichung, measurements of the four characters given below were linearly combined to make up a discriminant formula which maximized the variance among populations relative to that within populations, by the method described by Mather (1949). The formula thus obtained (standardized) was

$$X_3 = 1.00\,L + 0.64\,P - 0.67\,W - 0.09\,Aw,$$

where L, P, and W have the same signification as mentioned above and Aw represents awn length. Frequency distributions of the score given by this formula in various populations are given in table 3.

The data in table 3 show that the score varies among as well as within populations

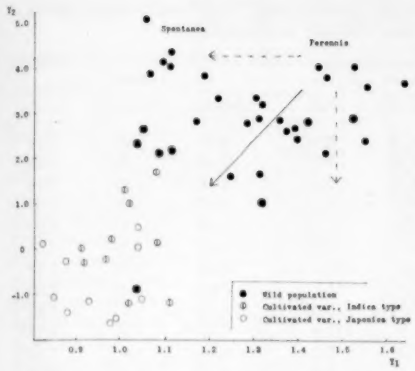

FIG. 4. Scatter diagram showing directions of differentiation. The abscissa and ordinate axes represent scores given by discriminant formulas for classifying *perennis* and *spontanea* types, and wild and cultivated types, respectively. Double circles show populations in rice fields or their proximity.

forming a continuous array of intergrades, while the populations classified as *spontanea* have smaller values and are accordingly located in the upper part of the table, above those classified as *perennis*. The rank correlation between X_3 and X_1, and that between X_3 and Y_1, were as high as 0.906 and 0.912, respectively.

Thus, mathematical treatments of the data by both principal component analysis and discriminant function method, consistently indicate as the principal direction of differentiation in this group the occurrence of *perennis* and *spontanea* types, though the variation between the two types is continuous. At the same time, it is interesting to find that the second axis indicates the tendency of wild populations to vary in the direction of the cultivated type.

For examining the relation between these two directions of variation, 32 wild populations and 20 cultivated varieties were scattered by the two discriminant functions already mentioned, Y_1 and Y_2. The scatter diagram thus obtained is shown in fig. 4. It shows that the populations intermediate between *perennis* and *spontanea* types tend to be distributed midway between wild and cultivated types, and if "highly wild" populations only are taken into account, the *perennis* and the *spontanea* types seem to be separated by a discontinuity in frequency distribution. It is also found from fig. 4 that the populations whose habitats were in rice fields or their proximity (indicated by double circles) are located close to cultivated varieties. This may be the outcome of introgressive hybridization, as pointed out by Oka and Chang (1959). Further, cultivated varieties of the Indica and Japonica types, classified by a discriminant formula presented by Oka and Fang (1957), are shown by different marks in the figure. The two types do not form their own clusters, however. The differentiation of cultivated varieties in relation to that between wild and cultivated types will be considered elsewhere.

3. *Intra-populational Variations as Compared with Inter-populational Ones*

It is known that wild populations of *Oryza* bear a large amount of genetic variability (Sakai and Narise, 1959; Oka and Chang, 1959). With the view to evaluating the relative magnitude of inter- and intra-populational variations in various characters, variance analysis was made using the data obtained in Taichung regarding a number of characters, and the genetic components of variances, σ^2_{Bt} (inter-populational variance) and σ^2_{Ws} (intra-populational variance), were estimated. The environmental variances to be subtracted were estimated on the basis of the values usually found for cultivated varieties, or those found in a pure line of wild rice (*spontanea* type) obtained from a haploid plant, or from the variance between replications.

From the results of this computation, the mean measurements for the *perennis*, *intermediate*, *spontanea*, and cultivated types, and t values for the differences between these types, are shown in table 4. The data in the table show that in spikelet width, grain weight, and anther length, *spontanea* is nearer the cultivated type

TABLE 4. *Mean measurements for* perennis, spontanea *and cultivated types and* t *values for the differences between them.* (Data obtained in Taichung)

Character	Mean measurements				*t* values[1] for		
	Perennis	*Interm.*	*Spontanea*	Cult.	*Per.–Sp.*	*Per.*–Cul.	*Sp.*–Cul.
Spikelet length (mm)	8.13	8.15	8.14	8.02	0.02	0.10	0.11
Spikelet width (mm)	2.27	2.55	2.56	3.05	1.10	1.89	1.18
100 grain weight (gm)	0.89	1.46	1.49	2.09	1.98	2.26	1.18
Awn length (cm)	5.87	8.31	6.91	0.72	0.37	1.70	3.02
Anther length (mm)	4.88	4.11	2.82	2.51	1.49	2.15	0.39
Ligule length (cm)	2.07	2.26	1.19	1.71	1.21	0.49	0.86
Plant height (cm)	150.2	125.2	84.5	141.7	2.10	0.30	1.63
Panicle length (cm)	21.3	16.7	13.3	21.8	2.00	0.30	1.80
Rachis no./panicle	7.20	6.63	5.06	10.20	1.28	1.05	1.80
Spikelet no./panicle	63.4	65.0	39.3	113.9	0.73	1.10	1.79
% of shedding	73.5	79.8	89.0	5.0	0.42	2.45	5.28
Dormancy of seeds[2]	2.19	2.21	2.12	1.16	0.19	2.91	3.74
$KClO_3$ resistance	2.71	2.57	2.65	2.26	—	—	—
No. of populations	11	10	14	85			
Av. plant no./popul.	23.2	28.1	34.3	5.0			

[1] $$t = \frac{M_1 - M_2}{\sqrt{\sigma^2_{Bt.1} + \sigma^2_{Bt.2} + \sigma^2_{Wn.1} + \sigma^2_{Wn.2}}}$$

[2] Shown by number of days required for "germinating activity" of hulled seeds to reach 40% and that of unhulled seeds to reach 20%, transformed in common logarithms and averaged.

than *perennis*; but in panicle length, plant height and some other characters, *perennis* is nearer the cultivated type than *spontanea*. It is also found that plant height, panicle length, grain weight, and anther length are the characters which differentiate the *perennis* and the *spontanea* types.

Intra-populational genetic variances found in the three wild types, and their ratios to the total genetic variances (sum of inter- and intra-populational genetic variances), are given in table 5. It is found in the table that in most characters, *perennis* populations had larger intra-populational variance than *spontanea* populations. On the contrary, *spontanea* populations tended to have larger inter-populational variances than *perennis* populations; the relative magnitude of inter-populational variance can be found from table 5 by subtracting the percentages of intra-populational variances from 100. In other words, it may be said that populations of the *perennis* type tend to store up genetic variations, while those of the *spontanea* type tend to vary in a wide range. An exception to this rule was found in awn length.

Genetic variation in a population can be apportioned into heterogeneity and heterozygosity. The genetic components of variance among plants of the original population ($\sigma^2_{p'}$), among line means of offspring (σ^2_p) and within lines (σ^2_0), and the covariance between parental plants and offspring lines ($\sigma_{p'p}$) were estimated from the results of variance analysis of the data obtained from plant-to-row experiments carried out in Taichung. The results for spikelet length, spikelet width, and apiculus hair length are given in table 6. As the data in the table show, in *perennis* populations, σ^2_0 was generally much greater than σ^2_p, but not always so in *spontanea* populations. Comparing the variance of parental plants $\sigma^2_{p'}$ with that of offspring $\sigma^2_p + \sigma^2_0$, it is found that the latter is larger than the former in *perennis* populations, but rather smaller in *spontanea* populations. These facts indicate that the genetic variability

TABLE 5. *Intra-populational genetic variances and their ratios to the total genetic variances in three wild types,* perennis, intermediate, *and* spontanea

Character	Intra-populational variance			Ratio in %[1]		
	Perennis	*Interm.*	*Spontanea*	*Perennis*	*Interm.*	*Spontanea*
Spikelet length (mm)	0.073	0.075	0.019	66	63	34
Spikelet width (mm)	0.023	0.005	0.003	56	21	16
100 grain weight (gm)	0.046	0.024	0.017	76	58	52
Awn length (cm)	0.979	0.808	0.757	15	16	57
Anther length (mm)	0.862	0.143	0.050	81	17	6
Ligule length (cm)	0.296	0.152	0.149	83	72	83
Plant height (cm)	262.1	144.2	96.8	91	23	14
Panicle length (cm)	6.80	2.48	1.10	65	47	19
Rachis no./panicle	1.084	0.692	0.303	70	31	15
Spikelet no./panicle	582.6	546.2	155.8	81	73	44
% of shedding[2]	739.6	826.9	431.9	98	67	68
Dormancy of seeds[2]	0.071	0.022	0.028	71	92	71

[1] $\frac{\sigma^2_{Wn}}{\sigma^2_{Bt} + \sigma^2_{Wn}}$ in % is shown.

[2] Phenotypic variances are shown.

in *perennis* populations is mainly due to heterozygosity.

4. *Variations in Photoperiodic Response*

Photoperiodic sensitivity and critical day-length of a population were estimated on the basis of difference in heading date between the plants seeded in Taichung on May 23 and June 22, as already mentioned. The sensitivity index measured by this method may be regarded as showing the requirement of the given plants regarding a certain critical day-length necessary for flower initiation. Among the populations investigated, those classified as *spontanea* generally showed earlier heading dates and accordingly longer critical day-length than those classified as *perennis*.

It has been shown (Oka, 1954, 1958b) that among cultivated varieties, the lower was the latitude of the distribution area, the shorter was the critical day-length. Whether such a latitudinal variation occurs among wild populations, or not, was then examined. The results proved, as shown in fig. 5, that the populations investigated could be divided into two latitudinal clines in both of which the lower was the latitude of the habitat, the shorter was the critical day-length; one of them was represented by *spontanea* and the other by *perennis* populations, the intermediate types being allocated to either of them. The cline of *perennis* populations had about 35 minutes shorter critical day-lengths than that of *spontanea* populations if those collected from the same latitude were compared.

Regarding the photo-sensitivity index, it was found that *perennis* populations were generally highly sensitive, while *spontanea* populations varied from medium to high sensitivity. A marked difference between the wild and the cultivated plants was the

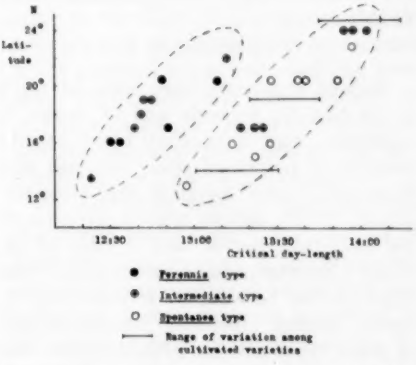

FIG. 5. Scatter diagram showing latitudinal clines of critical day-length.

TABLE 6. *Genetic components of variances and parent–offspring covariances of three characters estimated in populations of* perennis *and* spontanea *types*

Popul. no. and type	Collected from	Character[1]	Values of components estimated				
			$\sigma^2_{p'}$	$\sigma_{p'p}$	σ^2_p	σ^2_0	σ^2_e
W207	Cuttack,	Sp. Lth	.0522	.0274	.0490	.0756	.0954
Perennis	Orissa,	Sp. width	.0101	.0064	.0060	.0147	.0175
	India	Hair	.0735	.0396	.0645	.2233	.3694
W208	Raipur,	Sp. Lth	.1508	.1081	.0356	.2378	.0851
Perennis	Madhya P.	Sp. width	.0355	.0285	.0320	.0165	.0143
	India						
W201	Hooghli,	Sp. Lth	.0943	.0386	.0570	.0699	.0665
Spontanea	Bengal,	Sp. width	.0132	.0041	.0026	.0047	.0092
	India	Hair	.2765	.1589	.1019	.0477	.2367
W203	Cuttack,	Sp. Lth	.0526	.0125	.0172	.0155	.0584
Spontanea	Orissa,	Sp. width	.0133	.0042	.0094	.0013	.0173
	India	Hair	.0735	.0058	.0126	.0150	.2579

[1] Sp. Lth — Spikelet length in mm.
Sp. width — Spikelet width in mm.
Hair — Apiculus hair length in 0.1 mm.
$\sigma^2_{p'}$ — Genetic component of variance between plants of the original population.
$\sigma_{p'p}$ — Covariance between plants of the original population and offspring lines.
σ^2_p — Genetic component of variance between offspring lines.
σ^2_0 — Genetic component of variance within offspring line.
σ^2_e — Non-genetic component of variance between single measurements.

insensitivity of a part of cultivated varieties, though all wild populations were sensitive in varying degree. Among photosensitive cultivated varieties, the lower the latitude of distribution area, the higher is the sensitivity (Oka, 1954, 1958b). The same latitudinal variation was also found among wild populations, though a few *spontanea* populations were outside the cline. For details see Oka and Chang (1960).

DISCUSSION

Various arguments have been set forth as to how *O. perennis* and *O. sativa* f. *spontanea* may have contributed to the creation of cultivated rice: Sampath and Rao (1951) and Sampath and Govindaswami (1958) assume that *O. perennis* is the ancestral species, and a part of *O. sativa* f. *spontanea* has been produced from hybridization between *O. perennis* and *O. sativa*, because *spontanea* populations are of many types and are often found in the proximity of rice fields. On the other hand, Chatterjee (1951) accepts the view of Roschevicz (1931), that *O. sativa* f. *spontanea* may be the progenitor of cultivated rice in view of their similarity in various characters. However, the relationship between these two wild forms is not sufficiently accounted for, and neither of the arguments is supported by enough evidence to reject the alternative one.

The present study has proved that *O. perennis* and *O. sativa* f. *spontanea*, or the *perennis* and *spontanea* types of Asian wild rice, cannot be discriminated clearly by any one of the characters investigated alone, or by discriminant functions combining several of them to maximize the difference, insofar as our materials are concerned. However, the first principal component of the variation, mathematically extracted from our data, indicates the tendency for populations to be differentiated into the *perennis* and the *spontanea* types. The two types differ in habitat; *perennis* populations grow in deep-water swamps, while *spontanea* populations are found in marshy patches which may be parched in the dry

season. If the habitat is always submerged in water, plants once established may propagate asexually, while propagation by seeds may be to some extent hindered. If the habitat is dry once a year, seed propagation may be obligatory unless the plants can survive the drought. The perennial habit of *perennis* populations has been shown by their regenerating ability and the presence of partially fertile plants in them. Though the actual proportion of sexual and asexual propagations remains unknown, it may be assumed that the higher the frequency of partially fertile plants in a population, the higher would be the percentage of asexual propagation, because sterility would be eliminated in seed-propagated populations.

The difference between the two types in photoperiodic response also indicates a difference in ecological requirements. The occurrence of two parallel latitudinal clines for critical day-length indicates that in both types, heading at a proper time is strongly required, but there may be two modes of adaptation which constitute the difference between the two types. The high sensitivity to photoperiod of *perennis* populations may be due to their perennial habit, since if their sensitivity were low they would produce panicles continually and would be exhausted. The relatively low sensitivities of *spontanea* populations may be an adaptation to the annual fluctuations at the advent of the dry season, since a low sensitivity will bring about a non-heritable variation in heading date, and will enable a population to respond to such fluctuations in the environment. Thus, the *perennis* and the *spontanea* types seem to have different modes of adaptation to their habitats in accordance with their difference in the propagation habit.

According to Stebbins (1958), a constant swamp may be said to be a "stable habitat." Habitats that are parched in the dry season may be relatively unstable ones. Stebbins has shown with many examples that plant species growing in "stable habitats" are generally long-lived, and through cross fertilization store up a large amount of genetic variability, while those in "unstable" or easily disturbed habitats have an efficient method of seed propagation and a tendency to restriction of gene recombination, and are each adapted to a particular niche. A mathematical study by Dr. M. Kimura of our Institute (unpublished) also showed that a plant population of long life tends to be more heterozygous than an annual one. Our study shows that in most characters, populations of the *perennis* type have a larger amount of latent genetic variability, mainly in the form of heterozygosity, than those of the *spontanea* type, while those of the latter tend to vary in a wide range. As to their breeding systems, both these two types were found to be 30–40% allogamous (Oka, 1956; Oka and Chang, 1959; Sakai and Narise, 1959), though it is possible that the *perennis* type has a higher percentage of allogamy than the *spontanea* type (the writers' unpublished data). Thus, the differences between populations of the *perennis* and *spontanea* types seem to be in fair agreement with the view of Stebbins regarding the difference between species of "stable" and "unstable" habitats.

It may be taken for granted, as Stebbins (1958) assumes, that in higher plants, a species of stable habitats, which tends to be long-lived, out-crossing and rich in variability, is more likely the progenitor of related species than those of unstable habitats. In the light of this hypothesis, the *perennis* type of wild rice may be regarded as the ancestral form from which the *spontanea* type might have been derived. It may then be said, in view of the continuous variation between them, that *O. sativa* f. *spontanea* is a form of *O. perennis*.

We may then consider how cultivated plants can evolve from these wild forms. Assuming that *perennis* is the progenitor, our question is whether the cultivated type is derived directly from the progenitor, or by passing through the *spontanea* type. We can only imagine that the incipient cultivated genotypes might have arisen by mu-

tations and recombinations, due to a potential ability of the wild genotypes to produce them. Then the most essential factor in creating a cultivated type may be the occurrence of niches in which such plants have a selective advantage, for instance, a land cultivated or disturbed by man. In this process, if a wild population is of perennial habit, plants heterozygous for new genes may be maintained for many years, so far as they are recessive, and genic changes may be accumulated in the population. Thus, populations of the *perennis* type may store up the genetic materials necessary for creating cultivated genotypes and may wait for opportunities to have them tested.

In contrast, if a wild population is of annual habit, new genic changes will be tested in a few years. Therefore, only when new genic changes are continually supplied and the habitat assumes the conditions of cultivated land, the population genotype may approach a cultivated one. This process was demonstrated by Oka and Chang (1959), who investigated intermediate *spontanea*-cultivated populations found in the suburb of Raipur, India, which may have arisen by introgression of genes of cultivated plants and a "cultivation pressure" to the habitats. It seems that at the present time, wild plants of both *perennis* and *spontanea* types may contribute new variants to the cultivated rice, because the gene flow from cultivated plants is vigorous and niches under cultivation pressure are plentiful. But considering the earlier stage of development of the cultivated type, it seems plausible to assume that wild plants of the *perennis* type might have contributed more to the creative evolution than those of the *spontanea* type. It is however a possibility, in view of the tendency of intermediate *perennis–spontanea* populations to approach the cultivated rice, that cultivated types have been derived from such intermediate plants.

Populations intermediate between the wild and cultivated types may be regarded either as showing the potentiality of wild genotypes to produce them, or as due to introgression of genes of cultivated plants. However, it matters little, as to the significance of such intermediate populations, whether they have played a primary or secondary role in the historical sense, since the same forces may act in both "primary" and "secondary" processes. The results of this study suggest that the evolutionary change from *perennis* to cultivated rice might be due to two kinds of forces, so to speak, vectors, one directing toward the *spontanea* type, and the other toward the cultivated type, as shown by broken-line arrows in fig. 4. The external agent promoting the former process might be compulsory seed propagation, for instance, due to a topographic change in the habitat. The latter might be promoted by man, bringing about changes in characters differentiating the cultivated from the wild types, such as seed dormancy, grain shedding, etc. These two forces or vectors, if combined, may gradually produce plants approaching a cultivated type from wild plants.

Summary

Variations between and within populations of two wild rice types of Asia, *Oryza perennis* and *O. sativa* f. *spontanea*, were studied in an attempt to evaluate how they might have taken part in the creation of cultivated rice, *O. sativa*. A number of populations belonging to this group, collected from India, Thailand, and Malaya, were investigated regarding various morphological and physiological characters, in Misima, Japan (greenhouse and automatic shortday fields), and in Taichung, Formosa (experimental field). The populations formed a continuous array of intergrades, and the two types cannot be clearly discriminated. By using techniques of multivariate analysis, however, the first component of the variation was found to be given by the different character complexes of the *perennis* and *spontanea* types. It was also found that they might have different modes of adaptation to habitat related to differences in propagating habit;

perennis can propagate asexually, *spontanea* only by seeds. This difference might be the moving agent in effecting other differences, not only in various characters but also in the genetic structure of the *perennis* and *spontanea* populations. Populations of *perennis* type tend to store up genetic variability, while those of *spontanea* type tend to vary in a wide range. The second component of the multivariate variation indicates that cultivated types originated from wild ones. Populations intermediate between *perennis* and *spontanea* types tended to some extent to approach the cultivated type. In view of the tendency of *perennis* populations to store up genetic variability, this type may be the progenitor of both *spontanea* and cultivated types, and the evolutionary change from *perennis* to cultivated types may have been caused by two selective agents, obligatory seed propagation which directs a *perennis* population toward *spontanea*, and "cultivation pressure" which directs it toward the cultivated type.

Literature Cited

Chatterjee, D. 1948. A modified key and enumeration of the species of *Oryza* Linn. Ind. Jour. Agr. Sci., **18**: 185–192.

——. 1951. Note on the origin and distribution of wild and cultivated rices. Ind. Jour. Genet. & Pl. Breed., **11**: 18–22.

Fisher, R. A. 1936. The use of multiple measurements in taxonomic problems. Ann. Eugenics, **7**: 179–189.

Hector, G. P. 1935. Breeding and genetics of rice in India. Proc. World's Grain Exh. & Conf., Regina, Canada, 1933, **2**: 121–126.

Kendall, M. G. 1957. A course in multivariate analysis. Griffin's Statistical Monographs and Courses, 2.

Mather, K. 1949. Biometrical genetics. Metheuen & Co., London.

Morinaga, T., and H. Kuriyama. 1956. Cytogenetical studies on *Oryza sativa* L. VIII. The F_1 hybrid of *O. sativa* L. and *O. cubensis* Ekman. Jap. Jour. Breed., **6**: 133–141.

Nezu, M., T. C. Katayama, and H. Kihara. 1960. Genetic study of the genus *Oryza*. I. crossability and chromosomal affinity among 17 species. Seiken Ziho (Rep. Kihara Inst. Biol. Res.), **11**: 1–11.

Oka, H. I. 1953. Variations of various characters and character combinations among rice varieties. Jap. Jour. Breed., **3**: 33–43.

——. 1954. Varietal variation in the responses to day-length and temperature and the number of days of growing period. Jap. Jour. Breed., **4**: 92–100.

——. 1956. Observations on natural populations of Formosan wild rice. Ann. Rep. Nat. Inst. Genetics, Japan, **6**: 46–47.

——. 1958a. Intervarietal variation and classification of cultivated rice. Ind. Jour. Genet. & Pl. Breed., **18**: 79–89.

——. 1958b. Photoperiodic adaptation to latitude in rice varieties. Phyton, **11**: 153–160.

Oka, H. I., and C. C. Fang. 1957. Classification of rice varieties by discriminant functions. Jap. Jour. Breed., **6**: 41–44.

Oka, H. I., and W. T. Chang. 1959. The impact of cultivation on populations of wild rice, *Oryza sativa* f. *spontanea*. Phyton, **13**: 105–117.

—— and ——. 1960. Survey of variations in photoperiodic response in wild *Oryza* species. Bot. Bull. Acad. Sinica, **1(1)**: 1–14.

Roschevicz, R. J. 1931. A contribution to the knowledge of rice. Bull. Appl. Bot. Genet. & Pl. Breed., **27**: 3–133.

Roy, S. C. 1921. A preliminary classification of the wild rices of the Central Provinces and Berar. Agr. Jour. India, **16**: 365–380.

Sampath, S., and N. Rao. 1951. Interrelationships between species in the genus *Oryza*. Ind. Jour. Genet. & Pl. Breed., **11**: 14–17.

Sampath, S., and S. Govindaswami. 1958. Wild rices of Orissa—Their relationship to cultivated varieties. Rice News Teller, July 1958 issue.

Sakai, K. I., and T. Narise. 1959. Genetic variability in wild populations of *O. perennis* and *O. rufipogon*. Ann. Rep. Nat. Inst. Genetics, Japan, **9**: 66–70.

Stebbins, G. L. 1958. Longevity, habitat, and release of genetic variability in the higher plants. Cold Spring Harbor Symposia on Quant. Biol., **23**: 365–378.

Watt, G. 1891. Dictionary of the economic products of India, Vol. 5. (Quoted from Roschevicz 1931).

CHROMOSOMAL FITNESS CHANGES IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA PERSIMILIS* FROM TIMBERLINE IN THE SIERRA NEVADA[1]

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Much of the evidence for the adaptive function of *Drosophila* chromosomal polymorphism has been derived from experimental population cage analysis. Observation of changes in relative frequencies of competing chromosomal arrangements over a short term of about eight to ten generations is usually sufficient to establish determinacy of outcome and the net adaptive, or fitness, values of the competing karyotypes (Wright and Dobzhansky, 1946; Dobzhansky, 1948b; Dobzhansky and Levene, 1951). However experimental populations once initiated evolve new genotypes. With a given initial genetic variance the random mating population will seek its optimum genetic equilibrium within the limits of a constant laboratory environment. Such an equilibrium may or may not be close to that characteristic of the wild population from which the genotypes were derived, depending on the cleverness of the investigator at duplication of the conditions upon which the equilibrium is contingent. Of course since most of the factors of the wild environment upon which the population's adaptation depends are virtually unknown, the investigator can never hope to approximate the wild conditions; consequently the laboratory conditions are novel to the species, yet they are not so extreme that the species' norm of reaction cannot permit reasonable survival and then adjustment of its gene pool. It is this adjustment which must be taken into account in any long term analysis. Presumably in short term studies the changes in relative frequencies of competing genotypes tell us the immediate responses of a gene pool to the laboratory environment. Often these responses can be interpreted simply in terms of constant adaptive values throughout the range of changes, but several recent studies have shown such an interpretation to be too simple and not realistic for long term analysis.

Two important phenomena intrude upon the simple scheme of constant adaptive values for genetic variables in a constant environment: first, the phenomenon of frequency dependent values, or interaction between component genotypes (Levene, Pavlovsky, and Dobzhansky, 1954, 1958; Lewontin, 1955; Spiess, 1957); and second, the phenomenon of recombination in releasing variability and the production of new genotypes to fit the population more efficiently for a constant laboratory environment (Dobzhansky, 1954; Dobzhansky and Levene, 1951; Dobzhansky and Pavlovsky, 1953; Levine, 1955; Lewontin, 1958).

While one may observe changes in relative frequencies of genetic variables that may not fit a simple scheme of constant adaptive values, it is difficult to argue in many cases for conclusive evidence that one or the other of these phenomena bring about the noteworthy changes. It has been indicated by Lewontin (1957, 1958) that one could distinguish genotypic evolution throughout a long term population analysis by re-extracting the chromosomal arrangements and by sampling after a single generation populations with flies containing re-extracted chromosomes. It is to be hoped that such technique may be able to distinguish these two phenomena.

In this paper the original long term populations are to be described and the possibilities for interpretation based on com-

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parison of replicate populations will be discussed. Current experimental populations initiated with flies derived by re-extracting the relevant chromosomal arrangements at the point where the original populations were terminated will be described in a subsequent paper. The present paper has three objectives: (1) to present data in long term population cage analysis which might be resolved in terms of either the recombination and evolution of novel genotypes or frequency dependent adaptive values or both; (2) by employing stocks of *Drosophila persimilis* from Timberline in the Yosemite area of California to extend the population analysis of an altitudinal transect begun by Spiess (1950 and 1957); and thus (3) to compare laboratory population adaptive values with those values of karyotypes from lower elevations in the transect.

Timberline (the Stanford Experimental Garden at 10,000 ft) is about 25 miles east of the White Wolf locality (8,000 ft) (see Dobzhansky, 1948a). The commonest gene arrangement of chromosome III (Whitney) attains a very high frequency there: collections made by Dobzhansky at Timberline in 1946–47 (see *op. cit.* and Spiess, 1950) had about 92.0% WT, 2.7% MD (Mendocino), 1.3% KL (Klamath), and 4% ST (Standard) with a sample size of 74 chromosomes. In 1954 Dobzhansky collected there again and generously contributed the progeny of 33 wild females to our laboratory, with the following frequencies 87.5% WT, 6.7% MD, 5.0% KL, and 0.8% ST, figuring the sample size to be 132 chromosomes by consideration that the progeny of one wild female gives identification of four wild chromosomes. These figures are slightly higher in WT and MD than at the White Wolf locality (Spiess, 1957).

In view of the fact that laboratory populations containing the WT arrangement from White Wolf display balanced polymorphism with MD or KL as the WT becomes frequent, and that there is consequently evidence of a change in karyotype fitness as WT increases (especially in the WT–MD populations) (Spiess, 1957), it becomes essential to test other wild populations to compare the performance of WT again. In addition, the rare arrangements MD and KL from White Wolf showed no selective differences when WT was missing. It is expected logically that Timberline flies with karyotypes containing MD and KL should react similarly to White Wolf flies since these arrangements are rare at Timberline as well. Thereby the hypothesis can be tested that natural selection has had no opportunity to act upon the three karyotypes (MD/MD, KL/KL, and MD/KL) in these localities simply because they are so rare, and consequently if balanced polymorphism is acquired by selection through coadaptation, no adaptive equilibrium for these arrangements should be expected.

Materials and Methods

Populations of *D. persimilis* were initiated between 1956–57 in plastic cages at constant temperature (15° C). Progeny of flies collected by Dobzhansky in 1954 at Timberline were made homokaryotypic by single pair matings for 2–6 generations. Four different homokaryotype strains of each arrangement were used in equal numbers to initiate each population. Initial frequencies (from control egg samples) are given in table 1. For techniques of sampling cages, see Spiess, 1957. Chromosome samples usually were taken from 100 larvae each generation. At 15° C the average generation time per food creamer is ap-

Table 1. *Initial data for the experimental populations containing chromosomes from Timberline*

Cage no.	Date started	Total flies	Control Freqs.		
			% WT	% MD	% KL
30	Oct. 31, 1956	574	32.4	67.6	–
31	Jan. 4, 1957	601	26.5	73.5	–
32	April 22, 1957	521	40.0	–	60.0
32 Repeat	Oct. 17, 1957	537	35.5	–	64.5
35	Nov. 5, 1957	560	50.0	–	50.0
36	Oct. 24, 1957	560	55.0	45.0	–
37	Oct. 23, 1957	584	–	34.0	66.0
38	Oct. 30, 1957	585	–	66.0	34.0

TABLE 2. *Sampling data for cages containing WT and MD chromosomes*

Cage 30			Cage 31			Cage 36		
Sample	Days	% MD	Sample	Days	% MD	Sample	Days	% MD
			1	0	73.5	1	0	45.0
1	0	67.5	2	53	67.5	2	110	47.5
2	64	69.0	3	114	74.5	3	160	46.0
3	123	71.5	4	302	74.0	4	335	39.5
4	170	64.0	5	354	64.0	5	396	44.0
5	266	71.0	6	407	61.5	6	439	27.0
Terminated Aug., 1957			7	509	50.0	7	487	35.7
			8	669	47.0	8	583	32.5
			9	759	41.0	9	635	28.6
			10	813	45.0	10	735	16.0
			11	911	33.0	11	840	22.0
			12	1,009	45.5	12	939	16.0
			13[1]	1,188	40.5	Terminated June, 1960		
			Chromosomes Re-extracted April, 1960					

[1] Spontaneous inversion observed in MD/MD with breaks at 68 B/C and 81 C/B.

proximately 50 days. Food creamers were changed every four days so that with 16 creamers per cage, each creamer was kept in the cage 64 days, which allows plenty of time for emergence of the majority of flies, especially those flies developing from the first batch of eggs laid in the creamer.

POPULATION DATA

Whitney vs. Mendocino.—The frequency data of three populations (30, 31, and 36) containing WT and MD arrangements are given in table 2 and are shown graphically in fig. 1. In all three populations no significant changes occurred for six to eight generations. These populations might have been automatically terminated (indeed one was terminated) on the assumption that no further changes of interest would take place had it not been for the recent information from Lewontin (1958) and Levine and Beardmore (1959) with data on long term populations. It seems obvious from this constancy of initial frequencies that the immediate responses of zygotes containing WT and MD arrangements to the laboratory environment were equivalent. After this initial period however, MD fell off in both populations 31 and 36 at about

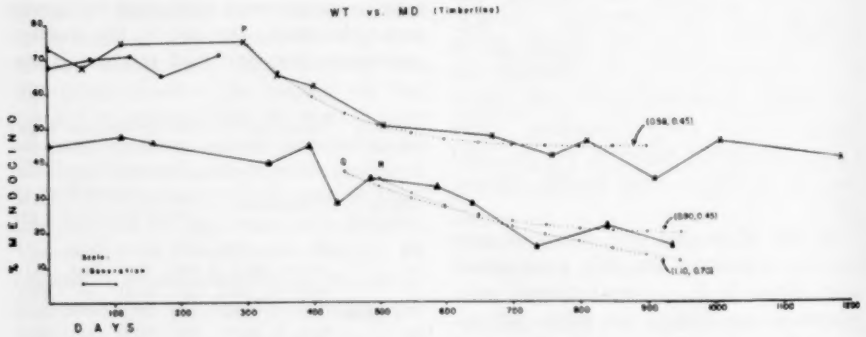

FIG. 1. Observed frequencies of MD in populations containing WT and MD chromosomes. Cage 30 = solid circles, cage 31 = X's, and cage 36 = triangles. Fitted curves dotted: fitness values respectively WT/WT, MD/MD, assuming WT/MD = 1.00.

TABLE 3. *Sampling data for cages containing WT and KL chromosomes*

Cage 32			Cage 32R			Cage 35		
Sample	Days	% KL	Sample	Days	% KL	Sample	Days	% KL
1	0	60.0	1	0	64.5	1	0	50.0
2	50	61.2	2	50	53.5	2	52	37.5
3	141	49.3	3	101	51.5	3	101	40.5
4	222	28.5	4	150	47.5	4	150	35.0
5	271	42.0	5	199	50.5	5	200	31.5
6	320	21.5	6	260	42.5	6	253	21.0
7	370	25.5	7	302	32.0	7	350	12.0
Terminated May, 1958			8	362	33.0	8	401	5.0
			9	438	22.5	9	507	3.5
			10	504	16.5	10	597	2.0
			11	560	14.0	11	647	2.7
			12	601	8.5	12	695	0.5
			13	652	4.0	13	745	2.0
			14	720	7.5	Terminated Dec., 1959		
			15	820	5.5			
			16	882	1.0			
			Chromosomes Re-extracted April, 1960					

the same overall rate, though the changes in 36 were less regular than in 31.

The fact that changes in these two populations were parallel and not converging with different final frequencies after some 1,000 days suggests that the genetic components of fitness have changed from the sixth generation onwards, and that such genetic changes have been different in the two cages. In cage 31 the change appears to be brought about by improvement in the WT/MD fitness and decline in MD/MD fitness relative to WT/WT. A hypothetical selection curve has been fitted (using methods of Dobzhansky and Levene, 1951; and Wright, 1946) to these observed changes from point "P" appearing as a dotted line in fig. 1. Cage 31 changes show a good fit with adaptive values of WT/WT = 0.58, WT/MD = 1.00, MD/MD = 0.45 coming to equilibrium at $q_{MD} = 0.43$.

Cage 36 is not easy to fit by any means to a single selection curve. The curve from point "*Q*" in fig. 1 (WT/WT = 0.90, WT/MD = 1.00, MD/MD = 0.45) may describe an overall change which may be the ultimate set of values after about the 750th day. The fit along most of the curve is not very satisfactory. Most of the trouble comes from the convexity between the 500th and 750th day, which is not typical of any selection curve produced by a single constant set of adaptive values. With an apparent equilibrium being attained at about $q_{MD} = 0.18$ from the 750th to 950th day, it is possible that the karyotypes have evolved these new adaptive values by that time. On the other hand, a curve (WT/WT = 1.10, WT/MD = 1.00, MD/MD = 0.70) has a slightly better fit from point "*R*" but will attain eventual elimination of MD. With this ambiguity in cage 36, it seems best to state the most likely possibilities: either (1) adaptive values are changing as new genotypes are being evolved so that no single set of values will fit the observed changes, or (2) the convexity comes from a random non-adaptive increase of MD which then falls rapidly back to a level determined by selective values.

While the attainment of a coadapted equilibrium in cage 36 would be of great interest (see discussion) the exact nature of the karyotype adaptive values is secondary to the fact that these data demonstrate an evolution of genic contents of chromosomal arrangements from a state of near neutrality. It seems unlikely that these changes can be due to some frequency dependent mechanism affecting adaptive value changes. Populations 31 and 36 differ markedly in final outcome, as would be

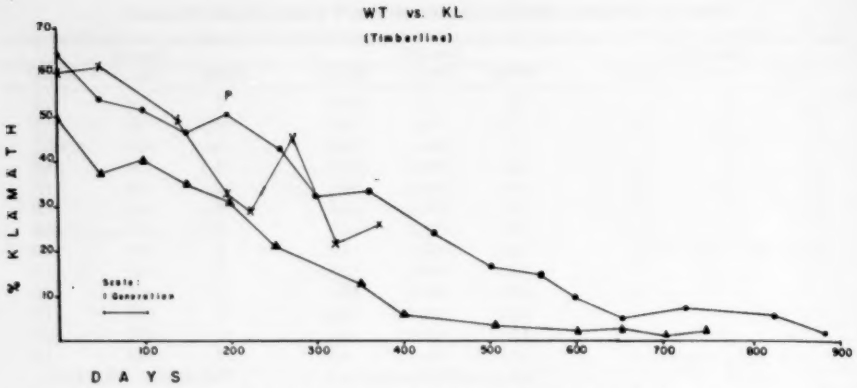

FIG. 2. Observed frequencies of KL in populations containing WT and KL chromosomes. Cage 32 = X's, cage 32Repeat = solid circles, cage 35 = triangles.

expected if high fitness is attained by different genetic systems in the two populations. Since all three populations were initiated with the same strains of WT and MD, the difference in outcome must result from the difference in original frequencies of the competing karyotypes and consequently the frequencies of recombinants potentially useful to the population.

Whitney vs. Klamath.—The frequency changes in three populations (32, 32R, and 35) containing WT and KL chromosomes are given in table 3 and graphically in fig. 2. All three populations agree in the tendency for KL to be eliminated at about the same rate, though fluctuations in cage 32 are large. In fig. 3 the observed curves for cages 32R and 35 are superimposed at point "*P*" on the assumption that their changes did not differ significantly from an average curve (dashed line) between them. By the methods of Levene and Wright the following adaptive values were calculated to fit this average curve: WT/WT = 1.50, WT/KL = 1.00, KL/KL = 1.00. This set of values gives no deviation greater than ± 0.009 from the average curve until the eleventh generation (table 4). Subsequently, however, the observed average line and both populations deviate increasingly in a positive direction from this expected curve because the latter falls below 0.02 on the twelfth generation and below 0.01 on the fourteenth generation. In view of

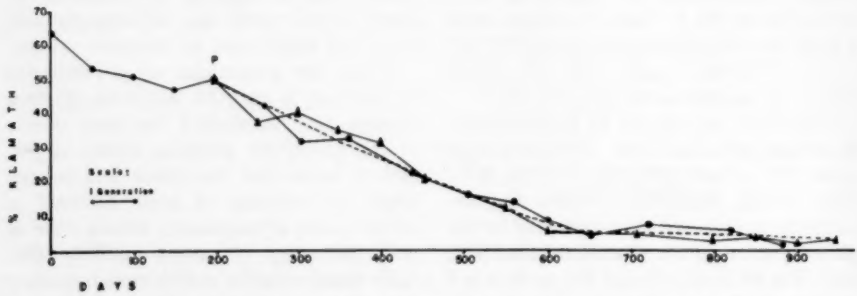

FIG. 3. Observed frequency changes in cages 32R and 35 superimposed at point "*P*" to give maximum agreement. Dashed line = "average," or smoothed, curve hand drawn to fit the selection curve from which fitness values were calculated (see table 4).

TABLE 4. *Observed average % KL of cages 32R and 35 from point "P" in fig. 3 and expected values for WT/WT = 1.50, WT/KL = 1.00, KL/KL = 1.00*

Generation	Observed Av. KL	Expected KL	Deviation
0	0.500		
1	0.440	0.444	−0.004
2	0.377	0.385	−0.008
3	0.320	0.324	−0.004
4	0.265	0.263	+0.002
5	0.215	0.207	+0.008
6	0.162	0.158	+0.004
7	0.117	0.116	+0.001
8	0.083	0.084	−0.001
9	0.057	0.059	−0.002
10	0.050	0.041	+0.009
11	0.043	0.028	+0.015
12	0.040	0.019	+0.021
13	0.037	0.013	+0.024

TABLE 5. *Sampling data for cages containing MD and KL chromosomes*

Sample	Days	Cage 37 % KL	Cage 38 % KL
1	0	66.0	34.0
2	71	68.0	–
3	124	71.0	36.5
4	223	55.0	–
5	337	41.5	35.5
6	386	54.7	33.3
7	488	46.0	26.5
8	586	39.5	17.0
9	636	30.0	10.0
10	676	27.5	13.0
11	739	23.0	15.3
12	840	19.3	6.8
13	942	13.0	8.0
14	1,039	22.5[1]	4.5

[1] Chromosomes re-extracted Sept., 1960.

this consistently positive deviation for at least four generations and the rather high accuracy of the rest of the curve, a reasonable interpretation might be that KL was being eliminated because of WT/WT superiority over the heterokaryotype until it approached a low frequency (3%) at which frequency it was being maintained. A change in adaptive values either brought about by genotype evolution or frequency dependency resulted in preserving the KL arrangement presumably by increasing the fitness of the heterokaryotype. Values approaching the following could satisfy this equilibrium: WT/WT = 0.99, WT/KL = 1.00, KL/KL = 0.66, with equilibrium $q_{KL} = 0.03$.

Again only by re-extracting chromosomes from the populations at their termination and reinitiating populations at high frequency of KL can some decision about these changes be made. At face value it would appear that no heterosis of the heterokaryotype occurs at intermediate frequencies of these gene arrangements, but that it is acquired in a degree sufficient to maintain a low frequency of KL either by genotype evolution through recombination and selection in this constant laboratory environment or by frequency dependent changes when WT becomes very common. The latter interpretation seems more likely because the probability for good agreement between independent populations (32R and 35) when new genotypes are evolving from different initial frequencies is certainly smaller than for good agreement between such populations affected by a constant frequency dependent change. Of course *a priori* reasons have often proved to be misleading in the past. Secondly, if the value of KL is actually an equilibrium reflecting the low frequency of the wild population at Timberline, the question arises as to an explanation for the completely unexpected neutrality of MD in competition with WT, in comparison. At present it might be more realistic to favor the view that for Timberline chromosomal arrangements the basis of adaptive control is too remote from the constant laboratory environment at 15° C, and that in neither WT–MD cages nor WT–KL cages are the immediate chromosomal responses imitating their responses to wild environment conditions.

Mendocino vs. Klamath.—The data from cages 37 and 38, in which initial frequencies of these arrangements are reversed, are given in table 5 and graphically in fig. 4. In contrast with the WT containing populations, the curves for populations 37 and 38 show less agreement and are more difficult to interpret. For two generations no significant change occurred in cage 37, then

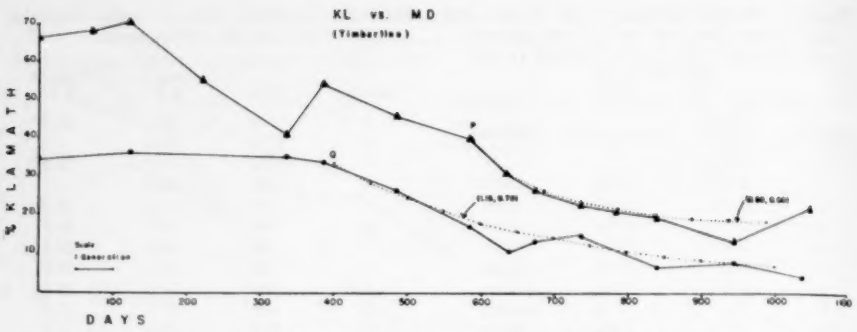

FIG. 4. Observed frequencies of KL in populations containing MD and KL chromosomes. Cage 37 = triangles, cage 38 = solid circles. Fitted curves are dotted with fitness values respectively MD/MD, KL/KL, assuming MD/KL = 1.00.

KL decreased to a level between 40–55% which was maintained with wide fluctuations for an additional eight generations (point "*P*" in fig. 4); and finally KL fell to a third level between 13–23%. Throughout these three phases the karyotype adaptive values must have changed considerably: initially all karyotypes about equal, then a lowering of KL/KL and probably an increase in the adaptive value of MD/KL relative to MD/MD, and ultimately a further lowering of KL/KL adaptive value to near lethality (see the dotted line from point "*P*" in fig. 4: MD/MD = 0.80, MD/KL = 1.00, KL/KL = 0.00 with equilibrium $q_{KL} = 0.17$).

Population 38 showed neutrality of karyotype fitness values initially for at least seven generations. Subsequently, however, a regular change took place different in outcome from that in population 37. In spite of a slight convexity between 650–850 days, the KL arrangement shows an overall drop leading to elimination. The dotted line in fig. 4 from point "*Q*" fits the observed curve fairly well: MD/MD = 1.15, MD/KL = 1.00, KL/KL = 0.70. With an intermediate heterokaryotype of course KL will be eliminated.

It may be expected that KL should be inferior to MD after comparing the results of populations containing WT: The original responses of WT vs. MD karyotypes were evidence of neutrality, consequently it might be expected that KL might compete with MD as it does with WT, or that MD vs. KL curves should duplicate WT vs. KL curves. The fact that KL does not decrease immediately but is neutral with MD at first demonstrates that the outcome of competition between the rare arrangements cannot be predicted from comparisons of populations containing the WT arrangement (as was the case with White Wolf MD vs. KL, see Spiess, 1957). Intrapopulational selection in a constant environment then must depend upon the specific chromosomal arrangement components introduced into that population.

Furthermore these long term populations containing the two rare arrangements which are hardly likely to have been coadapted in nature have evolved new genetic systems in these cages which are different in outcome: cage 37 coming to equilibrium at $q_{KL} = 0.17$ while cage 38 shows KL to be on an elimination course. The populations differ also in the rate at which new adaptive changes take place: in cage 37 after only two generations while in cage 38 after eight generations. As with populations containing WT–MD the reactions of these karyotypes are different because their initial frequencies were different since all strains used were identical and environmental conditions held constant.

Discussion

Population cage technique is useful but not always unambiguous in describing the net fitness of genetic variables competing in a population. Sample frequencies may vary from generation to generation because of sampling error (maximum about ± 0.07 with 95% confidence for frequencies close to 0.50), random fluctuations in competing genotypes, and selection determined parameters.

Random fluctuations in genotype frequencies present the greatest hazard to population cage interpretations. When indeterminacies, inconsistencies or deviations from a theoretical curve occur, some caution and weighing of evidence must be exercised. Comparisons of two or more populations may serve to decide whether a given fluctuation is of temporary nature or permanent. Such fluctuations, greater than sampling error, probably occurred in cages 36 (400–500 days), 32 (200–300 days), and 37 (300–500 days). Explanations for such deviations are not apparent, but they are clearly distinct from changes determined by long-acting selection since they usually return to a level determined by selection. Deviations from theoretical curves which persist over several generations and with uniform direction are more likely to be selection determined changes rather than random fluctuations.

There are at least three selection determined parameters which should be distinguishable by population cage technique: (1) constant fitness values for competing genotypes throughout the experiment, (2) fitness values which change owing to those values' dependence upon frequency of the genotypes, and (3) fitness values which change owing to evolution of new genotypes and new adaptive equilibria which raise the total fitness of the laboratory population above its initial response level. If a sufficient number of replicate populations could be maintained, one might be able to distinguish these three phenomena by the following comparisons respectively (assuming the populations are always initiated with the same strains but with different frequencies of competing genotypes): (1) uniformity of pattern with a regular series of changes fitting a simple selection curve and in addition convergence of different frequency curves upon one average curve, (2) uniformity of pattern between populations but irregular changes recurring at the same frequencies in each population, and (3) diverse patterns in the populations with different final equilibria. With limited space and time the investigator usually relies on two or three replicate populations for such comparisons. Consequently, to be more certain of the nature of the changes occurring, the extra chore of re-extracting homozygous strains and reinitiating populations is necessary.

Perhaps all three of these selection determined parameters may be illustrated by the data on Timberline populations presented above. The short term responses of populations containing WT show uniformity of pattern at first, one set showing strong selection differences (WT–KL) and the other set showing no selection differences (WT–MD). A frequency dependent change may take place in the WT–KL populations when KL reaches low frequency (5% or less) bringing about a low equilibrium for that pair of arrangements. Finally the diversity of pattern presumably brought about by genotype evolution after long periods seems to take place in the WT–MD populations but at different times in the MD–KL populations.

The immediate responses of these Timberline chromosomes to the laboratory environment are in very marked contrast to those of chromosomes taken from the White Wolf locality. From the first six to eight generations there was no evidence of heterokaryotype superiority in populations containing the WT arrangement from Timberline (Spiess, 1959). In fact, heterokaryotype superiority for WT/KL or WT/MD has never been lacking in populations of *D. persimilis* from lower elevations (White Wolf and Jacksonville, California) studied

at 15°–16° C. All the initial responses of Timberline chromosomes are different from those of White Wolf chromosomes. Consequently the same chromosomal arrangements even though separated by a short distance (25 miles and 2,000′ altitude) control different adaptive complexes and may therefore be racially distinct, re-emphasizing the fact that each local population consists of integrated genetic systems maintained by the selective influences of that particular local environment, as expressed by Mayr (1945) and Dobzhansky (1948b). Proof of racial distinctness would of course necessitate replication of these experiments with larger samples of chromosomes.

An agreement between population cage frequencies and wild population frequencies can hardly be hoped to be reached in view of the novelty that the population cage environment presents to the species. Superficially the WT–KL cages might not seem far from such agreement: the final frequency of KL is 3% in both cage and wild, but the experimental curve approaching that frequency is almost certainly an elimination curve for KL. If this proves to be a real case of frequency dependent change in adaptive value for the heterokaryotype WT/KL at low frequency, a possible parallel with the wild situation would be truly amazing. In view of the completely unexpected neutrality of WT–MD karyotypes in population cages however, it would be safer to assume that in neither case are adaptive values in cages imitating adaptive values in nature.

The unexpected responses of WT–MD karyotypes can only indicate the extreme novelty of population cage conditions, and it would not be useful to speculate at present about the possible environmental forces which might evoke adaptive responses in the laboratory akin to those in the wild.

Dobzhansky (1954, 1957) has demonstrated that natural selection may evolve new adaptive genotypes in laboratory populations of *D. pseudoobscura* containing novel genetic combinations, that is, mixtures of chromosomes from widely separated localities. In contrast with the uniform responses and determinacy of outcome characteristic of populations containing chromosomes from a single locality, these populations containing individuals descended from diverse wild populations responded in some cases by developing heterokaryotypes with superior fitness and in other cases by eliminating one of the chromosomal variants. To *D. persimilis* from Timberline, laboratory conditions are so novel that considerable gene pool alteration must occur by recombination and redistribution of genic contents within each karyotype. Dobzhansky produced indeterminancy of outcome by mixing genic competitors which had not been coadapted in nature. A similar indeterminate effect then is produced in Timberline *D. persimilis* populations because the environment is too novel for the coadapted system. This is probably true for the WT vs. MD and MD vs. KL populations but doubtful for the uniform WT vs. KL populations. It is worth noting that those populations which showed such diverse final frequencies displayed no selective differences initially: WT–MD populations with expectation for initial heterosis developed it eventually, probably in both cases; MD–KL populations with no expectation for initial heterosis developed it in one population but probably not in the other.

Lewontin (1958) proposed that "the maintenance of balanced polymorphism in a population is contingent upon environmental variability" because chromosomal polymorphism has been shown to provide a homeostatic device in a fluctuating environment. The converse might be true, according to Lewontin, that in a constant environment superior homozygotes may arise and substitute for superior heterozygotes with accompanying loss of balanced polymorphism. If however the balanced polymorphism confers a higher net fitness upon any population irrespective of the environment's fluctuating or constant prop-

erties, then such a converse statement would not necessarily apply. Beardmore, Dobzhansky, and Pavlovsky (1960) have demonstrated that under constant laboratory conditions greater biomass and consequently higher net fitness is characteristic of polymorphic in contrast with monomorphic populations of *D. pseudoobscura* containing AR and CH chromosomes from Pinon Flats, California. Lewontin drew conclusions from populations containing AR and PP chromosomes from Texas which came to a temporary equilibrium at about 85% AR but subsequently attained levels of 92–95% AR. He interpreted this change as an increase in the fitness of AR/AR relative to AR/PP and PP/PP, leading to elimination of PP chromosomes from the population. While the change in fitness was certainly demonstrated, the destination of monomorphism may be in doubt. As pointed out by Levine and Beardmore (1959), AR–PP (Texas) populations in Dobzhansky's (1957) laboratory also showed a loss of PP to about 5% frequency after 600 days. It is entirely possible that the PP may be retained in the population at that low frequency indefinitely. These frequencies attained by AR–PP competition are just the reverse of those attained in nature, as Lewontin indicates, a fact that parallels the results of these Timberline *D. persimilis* populations to which the laboratory environment is far too novel to evoke frequency changes in the direction expected from wild population frequencies. That the constancy of the laboratory environment is not critical in the retention or elimination of polymorphism is strongly indicated by the data of Levine and Beardmore (1959). Using AR chromosomes of California origin and CH chromosomes from Mexico, they showed that balanced polymorphism once attained in the constant environment was not lost after two years, emphasizing "that ample genetic variance and constant environmental conditions do not of themselves produce monomorphism from polymorphism."

In the Timberline *D. persimilis* populations heterosis is apparently gained rather than lost in cage 31 (WT–MD) and in cage 37 (MD–KL), probably in cage 36 (WT–MD), and possibly in the other cages as well. In no population was the low frequency chromosome actually lost or depressed below a measurable frequency in any generation even after twenty generations. This evidence together with that cited above would indicate that balanced polymorphism on chromosome III in either of these two species may confer higher net fitness upon the population than monomorphism irrespective of the environment's fluctuating or constant properties. Heterozygote superiority may be conferred upon many phenotypic qualities only one of which is the homeostatic quality. There is no doubt that homeostasis would be advantageous to a population trying to survive in a variable or oscillating environment. In spite of the investigator's attempts to keep laboratory conditions "constant," however, there is much evidence that a variety of niches does exist in experimental population cages. Attainment of chromosomal adaptive polymorphism may be a response to such variation within the closed system.

Summary

1. Experimental populations of *Drosophila persimilis* from Timberline in the Yosemite region of California containing two gene arrangements of the three most common arrangements of chromosome III were sampled for twenty or more generations at 15° C in plastic population cages.

2. Three populations of Whitney vs. Mendocino (WT–MD) show no significant changes for 6 to 8 generations, at which time one population was terminated; consequently the immediate response of these karyotypes is equality of fitness to the laboratory environment. In the two long term populations MD decreased, in one case to an equilibrium at about 43%MD, in the other to a possible equilibrium at 18% MD. These changes are interpreted as evi-

dence for the evolution of genic contents within the structural arrangements after the eighth generation. Since all populations were initiated with the same strains but in different frequencies, difference in outcome must reflect different frequencies of recombinants potentially available to the population.

3. Three populations of Whitney and Klamath (WT–KL) agree in the tendency for elimination of KL. The rate of KL loss corresponds to an elimination curve which would bring the KL frequency to less than 1% at the fourteenth generation. However KL is maintained at a value 2–3% above that expected for at least four generations. Possibly a change in fitness values occurred brought about either by genotype evolution or frequency dependency. The latter is more likely because of the close agreement between independent populations.

4. About 15% of wild flies at Timberline are WT heterokaryotypes and lack of heterosis for them in the immediate responses of these populations is unexpected. This result implies that the basis for adaptive control of these arrangements is not evoked by the constant laboratory conditions which are considerably remote from wild environmental conditions. Changes subsequent to the immediate response then are due to adjustment of the population's gene pool to laboratory conditions. These changes produce a diversity of results between populations which were initiated with the same strains but different frequencies of the competing arrangements.

5. Two populations containing MD–KL karyotypes agree initially and with expectation in showing no significant changes as an immediate response to laboratory conditions. Subsequently the population with high KL tends to lose KL in two stages until an equilibrium is attained at 17%KL. In the other population initiated with low KL that arrangement tends to be eliminated as evidenced by its continual drop up to the point of termination. The fact that KL does not decrease but is neutral with MD initially demonstrates that the outcome of competition between the rare arrangements cannot be predicted from comparisons of populations containing the WT arrangement. The two rare arrangements, hardly coadapted in nature, have evolved new and diverse genetic systems in the laboratory.

6. All initial responses of Timberline gene arrangements differ markedly from the responses of White Wolf arrangements, emphasizing that different genetic adaptive complexes of racial distinctness have been selected in local environments separated only by 25 miles and 2,000 ft elevation.

7. The fact that superior fitness of heterokaryotypes has been synthesized in at least two and possibly three of the long term populations indicates that constancy of a laboratory environment does not exclude the maintenance nor establishment of chromosomal polymorphism.

Literature Cited

Beardmore, J. A., Th. Dobzhansky, and O. A. Pavlovsky. 1960. An attempt to compare the fitness of polymorphic and monomorphic experimental populations of *D. pseudoobscura*. Heredity, **14**: 19–33.

Dobzhansky, Th. 1948a. Genetics of natural populations. XVI. Altitudinal and seasonal changes produced by natural selection in certain populations of *D. pseudoobscura* and *D. persimilis*. Genetics, **33**: 158–176.

——. 1948b. Genetics of natural populations. XVIII. Experiments on chromosomes of *D. pseudoobscura* from different geographic regions. Genetics, **33**: 588–602.

——. 1954. Evolution as a creative process. Caryologia, **6**: 435–449.

——. 1957. Mendelian populations as genetic systems. Cold Spring Harbor Symposium, **22**: 385–393.

—— and H. Levene. 1951. Development of heterosis through natural selection in experimental populations of *D. pseudoobscura*. Am. Nat., **85**: 247–264.

—— and O. Pavlovsky. 1953. Indeterminate outcome of certain experiments on *Drosophila* populations. Evolution, **7**: 198–210.

Levene, H., O. Pavlovsky, and Th. Dobzhansky. 1954. Interaction of the adaptive values in polymorphic experimental populations of *D. pseudoobscura*. Evolution, **8**: 335–349.

——. 1958. Dependence of the adaptive values of certain genotypes in *D. pseudoobscura* on the composition of the gene pool. EVOLUTION, **12**: 18–23.

LEVINE, L. 1955. Genotypic background and heterosis in *D. pseudoobscura*. Genetics, **40**: 832–849.

—— AND J. A. BEARDMORE. 1959. A study of an experimental population in equilibrium. Am. Nat., **93**: 35–40.

LEWONTIN, R. C. 1955. The effects of population density and composition on viability in *D. melanogaster*. EVOLUTION, **9**: 27–41.

——. 1957. The adaptations of populations to varying environments. Cold Spring Harbor Symposium, **22**: 395–408.

——. 1958. Studies on heterozygosity and homeostasis II: Loss of heterosis in a constant environment. EVOLUTION, **12**: 494–503.

MAYR, E. 1945. Symposium on the age of the distribution pattern of the gene arrangements in *D. pseudoobscura*: Some evidence in favor of a recent date. Lloydia, **8**: 69–83.

SPIESS, E. B. 1950. Experimental populations of *D. persimilis* from an altitudinal transect of the Sierra Nevada. EVOLUTION, **4**: 14–33.

——. 1957. Relation between frequencies and adaptive values of chromosomal arrangements in *D. persimilis*. EVOLUTION, **11**: 84–93.

——. 1959. Lack of heterosis in experimental populations of *D. persimilis* from Timberline in the Sierra Nevada. Genetics, **44**: 537.

WRIGHT, S., AND TH. DOBZHANSKY. 1946. Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *D. pseudoobscura*. Genetics, **31**: 125–156.

A COMPARISON OF THE NINHYDRIN POSITIVE AND PHENOLIC SUBSTANCES OF PAROTOID GLAND SECRETIONS OF CERTAIN *BUFO* SPECIES AND THEIR HYBRIDS

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Within the past few years the technique of paper chromatography has allowed certain new experimental approaches in systematics and genetics. This subject has been reviewed quite recently by Buzzati-Traverso (1960). Turner and Alston (1959) studied a natural hybrid population in the legume genus *Baptisia*, together with the parental species, and by means of chromatographic techniques showed clear recombination of species-specific phenolic and fluorescent substances in the hybrids. Furthermore, the parental, as well as other, *Baptisia* species could be certainly identified from the chromatograms alone. It is evident that this type of approach provides a valuable means of studying natural populations, from both genetic and evolutionary aspects, with favorable material. A prerequisite, of course, is the presence of a reservoir of genetically governed biochemical differences amenable to chromatographic analysis.

This investigation was initiated to explore the possibilities of using similar methods with animals. The toad genus, *Bufo*, was selected as the subject for several reasons; extensive investigations of speciation in *Bufo* are being conducted at The University of Texas, adequate material was available including numerous species and their hybrids, and the animals were maintained under controlled conditions, particularly with respect to diet. Although a few chromatograms of tadpoles were tried, by far the best results came from use of the secretion of the parotoid glands. Previously, Mr. B. J. Wilks had used parotoid gland secretions for chromatographic studies and had noted differences among *Bufo* species (unpublished results). All subsequent remarks relate to properties of these parotoid secretions.

MATERIALS AND METHODS

Fourteen species and certain of their hybrids were examined, but the major emphasis was upon three species: *Bufo woodhousei*, *B. speciosus* and *B. cognatus*. Hybrids of *woodhousei* × *speciosus* and *woodhousei* × *cognatus* were compared with their parental species. Two of these species, *cognatus* and *speciosus* (= *compactilis* in part of earlier publications) have been placed in one phyletic series (species group), while *woodhousei* has been placed in another group on evidence of morphology, mating call, and genetic incompatibility and hybrid sterility (Blair, 1956, 1959). Therefore, another objective of the present investigation was to gather additional data which might be pertinent to the systematic relationships of the species.

The toads varied in age from six months to about 3 years, averaging about two years. There was no indication that age was directly related to any variations in patterns observed, and *woodhousei* × *speciosus* hybrids aged 1 year and 2 years were identical. The diet included earthworms, grasshoppers, pill bugs, and meal worms. All toads were offered the same diet, though it is possible that certain species exhibited feeding preference. Kirk, *et al.* (1954) who studied fluorescent patterns of chromatograms of several species of land snails found that age, geographical location, and diet (using 3 alternative diets)

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had no effect upon the patterns. There is no reason to conclude, however, that diet would have no influence upon the compounds in the parotoid gland secretions though it may be minor.

Extracts from the parotoid glands were prepared as follows. The area around the gland was washed and dried and the secretion was pressed out, using clean rubber gloves, and absorbed with small rolled cotton balls (± 4 mm in diameter). Both glands were utilized, and the toad was unharmed. An obvious advantage of the method is that a series of chromatograms of the same individual may be analyzed. The cotton was dropped into a test tube and 0.2 ml of 75% ethanol was added. Efficient extraction had occurred in 2 hours though often extraction proceeded overnight. After setting overnight a gelatinous mass appeared, impregnating the cotton, but the gel did not apparently affect the substances being chromatogrammed.

For chromatography, descending chromatograms were run, using two different solvents, in one dimension only. The extracts were not reduced in volume, as they were so concentrated that fifteen applications sufficed. The solvents used were: (1) *n*-butanol, pyridine, water (1 : 1 : 1 v/v); (2) *n*-butanol, acetic acid, water (4 : 1: 1 v/v). The solvents were equilibrated overnight; the paper was placed in the chamber two hours before the run commenced, and the solvents were never reused. Spraying reagents utilized were: ninhydrin and diazotized *p*-nitroaniline. The latter is a general detecting agent for phenols, and colors develop quickly at room temperature.

Nine individual toads of each of the three species selected for major emphasis were used and as many hybrids as were available: 7 of *B. woodhousei* × *B. speciosus* (6 from a single cross and one from another cross), and 3 from *B. woodhousei* × *B. cognatus*. Regrettably, in the present investigation the actual parents of the hybrids utilized were no longer available. Obviously, conclusions from the results obtained can be drawn only within limitations imposed by the conditions noted. It is evident that in the future the actual parents should be included insofar as this is possible.

Results

The patterns obtained with ninhydrin will be discussed first. In the preliminary stages, when a number of different species were being compared, it was soon evident that rather striking differences in the "amino acid" patterns existed between some species. However, when ten individuals of *Bufo terrestris* from different locations were compared, nearly as great differences among toads of the same species appeared. This was quite unexpected, and in sharp contrast to the situation encountered in fluorescent compounds in *Baptisia*. In fact, previous experience, which includes a number of comparative studies of amino acid patterns (Alston and Irwin, 1961) had shown that amino acid patterns of different individuals and, indeed, different species are notably similar, even though it is well-known that free amino acid patterns are particularly sensitive to environmental factors (Possingham, 1956). Although *B. terrestris* patterns were somewhat more variable than those of certain other species tested, all species showed some individual variation. The three species of principal interest in this investigation showed far less individual variation in ninhydrin sensitive patterns than did *B. terrestris*, but also they showed rather little interspecific variation.

In solvent (1), which gave slightly better separation of the ninhydrin positive components, patterns of all three species were quite similar. *B. woodhousei* had fewer spots than the other two species, the average number of definite purple spots being six. In *B. cognatus* usually seven or eight spots appeared, and *B. speciosus* yielded seven or eight spots. However, the most prominent spots in all three species were grouped in the general area between Rf = 0.05 and 0.40, and the numerical differences convey an exaggerated conception

of the differences. The patterns of *B. cognatus* showed the least individual differences. In addition to the typical "amino acid" response to ninhydrin (i.e., purple coloration), at around Rf 0.80, all chromatograms of all species contained one (in *B. cognatus*) or two (in about half of the individuals of the other two species) bright blue-gray spots which faded to a straw color after about 24 hours. *B. woodhousei* and *B. speciosus* differed from each other as follows: in *B. speciosus* all toads contained the upper blue-gray spot and only six the lower; in *B. woodhousei* all toads contained the lower and five contained the upper. Among the hybrids of *B. woodhousei* × *B. cognatus*, all showed a single straw colored spot, typical of some of both parents. Certain of the hybrid's patterns could not be fully interpreted because of streaking; in solvent (1) only, *B. speciosus* and especially its hybrids showed a white opalescent smear near the bottom of the chromatogram which we interpreted intuitively as a probable cause for some of the smearing of these chromatograms.

In solvent (2), the "amino acid" spots were not significant because of the high degree of intraspecific variability superimposed upon relatively little interspecific difference in patterns. All individuals of all species and all the hybrids showed a single straw colored spot in solvent (2) at Rf = 0.45–0.05. Apparently the two bands of certain individuals of *B. speciosus* and *B. woodhousei* run as one band in this solvent. Thus, solvent (2) alone would not have exposed that rather striking variation which is noted with solvent (1).

Another feature of the solvent (2) ninhydrin patterns was the appearance of a cream colored spot just above the straw colored spot in a number of cases. In contrast to the straw colored spot, which is initially bright blue-gray, the cream spot is initially cream colored and does not change. Hence the two cannot possibly be confused. The distribution of this spot is very interesting. It is present in most of the series of *B. woodhousei*, but it is either very dilute or of only moderate intensity. Four individuals of *B. woodhousei* have another cream spot (of identical color) above the first. Only one other toad, a *woodhousei–speciosus* hybrid, showed this second cream component, and this case is doubtful. Seven of the *B. speciosus* series had the major cream component. It was definitely absent in three, rather dilute in three, moderately concentrated in one and concentrated in three. *B. cognatus* patterns, except one which was generally less concentrated, contained a small amount of the cream component. It reached moderate concentration in only one extract. In all the hybrids, however, the cream component was concentrated. In fact, the concentration of this component reached by all of the hybrids was attained in only three or four of the 28 parental types.

From comparison of the ninhydrin patterns, it was not possible to demonstrate recombination of parental components in the hybrids, though there is no reason to suspect that such did not occur.

The diazotized *p*-nitroaniline patterns, indicative of phenols, were particularly interesting. A number of substances were present which gave well-defined spots in the solvents used. Because of their intensity of color and regular occurrence certain reddish-lavender spots were exceptionally prominent. As in the case of the ninhydrin patterns, a certain amount of intraspecific variation occurred in the phenolic patterns although some species characteristics appeared to be constant. After comparison of a number of duplicate chromatograms, one of which was sprayed with ninhydrin, the other with the phenolic detector, it was evident that certain of the reddish-lavender phenolic spots were identical with the straw colored ninhydrin spots. These include the straw colored component of *B. cognatus* and two of the straw colored components of the other two species. However, certain *B. woodhousei* individuals showed three reddish-lavender spots in the butanol : pyridine : water solvent (see fig. 1), and

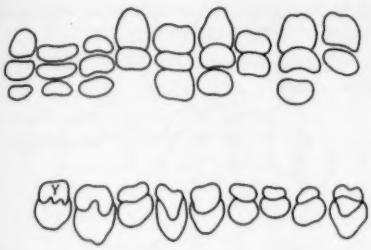

FIG. 1. Chromatogram, of 9 individuals of *B. woodhousei*, developed in *n*-butanol : pyridine : water (1 : 1 : 1) and sprayed with phenolic reagent. Note that all individuals exhibit two or three bands.

FIG. 2. Chromatogram, of 9 individuals of *B. cognatus*, developed in *n*-butanol : pyridine : water (1 : 1 : 1) and sprayed with phenolic reagent. Y represents a bright yellow band present in all individuals.

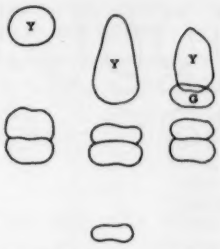

FIG. 3. Comparison of chromatogram of *B. woodhousei* (left), *B. cognatus* (right), and a *woodhousei* × *cognatus* hybrid (center). The upper band in each case represents the yellow component. It is not clear whether the hybrid contains only the "lower" yellow component or, in addition, some of the "upper" yellow component. The second from the top band in *B. cognatus* represents the "gray" component (G) described in the text. The lowest band is the spot designated "RL 0.25" in table 1. The solvent in this case is *n*-butanol : acetic acid : water (4 : 1 : 1).

only two straw colored spots with ninhydrin in the same solvent, so at least one of these compounds is not detected by ninhydrin. The cream component appearing with the ninhydrin treatment is also colored reddish-purple by the phenol detecting spray. This represents a very interesting situation, particularly in view of the fact that the substances may also be giving a positive Dragendorff test (for alkaloids). This last information is based upon chromatograms run in different solvent systems, and we cannot be absolutely certain that they are, in fact, the same substances. However, a group of indole derivatives known as bufotenines, found in parotoid secretions of several other toad species, contain phenolic and basic nitrogen groups, and it may be that compounds of this type are producing the reddish-lavender spots (Deulofeu, 1948).

There are species differences in the reddish-lavender components. As already noted in the discussion of the straw colored compounds, all individuals of *B. cognatus* produce only one straw colored spot while some of the individuals of the other two species produce two. All hybrids of *B. woodhousei* × *B. cognatus* produced one spot, while all hybrids of *B. woodhousei* × *B. speciosus* produced two spots. *Bufo woodhousei* is the only species which showed three reddish-lavender bands in solvent (1) (cf. figs. 1 and 2).

In hybrids of *B. woodhousei* × *B. cognatus* the single reddish-lavender spot which appeared on chromatograms run in solvent (1) ran at the Rf of the *upper* spot of *B. woodhousei*. In solvent (2) this spot separated, with the lower, more concentrated, component running at the same Rf as the *lower* spot of *B. woodhousei* (fig. 3). Thus, there is evidence that the relative positions of the reddish-lavender components differ in the two solvents used. Since the cream component is never seen in solvent (1), and since only one reddish-lavender band appears in *B. cognatus* individuals with this solvent, the cream component must be masked in this case by the straw colored component when sprayed with ninhydrin.

A much weaker reddish-lavender spot, not previously mentioned, running at Rf 0.20 in solvent (2) and Rf 0.45 in solvent (1), is present in most individuals of all three species and in all the hybrids.

In solvent (2) only, a distinct, gray spot appeared at Rf 0.70, after spraying with the phenolic detecting reagent. This spot was present in all *B. cognatus* individuals

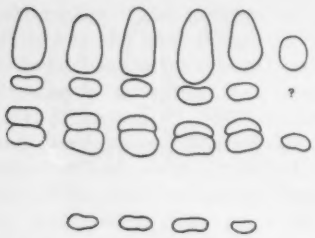

FIG. 4. Chromatograms of 6 individuals of *B. cognatus* developed in *n*-butanol : acetic acid : water (4 : 1 : 1). Question mark indicates position of gray component which is not definitely seen in one toad. However, all bands were less concentrated in this particular specimen. The lowest band, present in 4 individuals, is RL 0.25 .

and in varying amounts in five of the nine individuals of *B. speciosus* (figs. 4 and 5). However, it could not be certainly identified in *B. woodhousei* (a slight discoloration seemed to be present at the same Rf in two extracts, fig. 6). All hybrids of *B. woodhousei* × *B. speciosus* contained the gray component (fig. 7). It was not present in *B. woodhousei* × *B. cognatus* hybrids.

One other quite definitive interspecific difference in phenols was noted. In this case, a lemon-yellow substance (Rf = 0.95 in butanol : acetic acid : water) was present in *B. woodhousei* (definitely in 3); in *B. speciosus* (definitely in 3); and in high concentration in all seven of their hybrids (figs. 5, 6, and 7). This substance

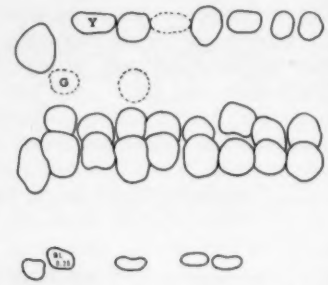

FIG. 6. Chromatograms of 9 individuals of *B. woodhousei* developed in *n*-butanol : acetic acid : water (4 : 1 : 1).

was not detected, however, in *B. cognatus*. Instead, a compound with the same color but running consistently at a lower Rf (Rf = 0.75) was present in *B. cognatus* and all of the *B. woodhousei* × *B. cognatus* hybrids (fig. 4). Examination of *B. cognatus* chromatograms run in solvent (1) disclosed a yellow component, not evident in the other species, which partially overlapped the reddish-lavender spot (fig. 2). It was not definitely established whether the two yellow spots were present together in the *B. woodhousei* × *B. cognatus* hybrids.

Table 1 summarizes the distributions of the "phenolic" compounds considered. In certain cases blanks occur, as in one *B. woodhousei* × *B. cognatus* hybrid. In these situations, either the chromatogram necessary to yield the information was not run

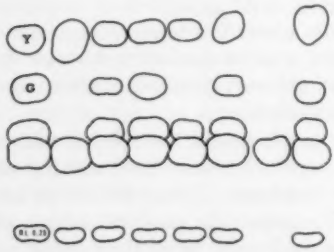

FIG. 5. Chromatograms of 9 individuals of *B. speciosus* developed in *n*-butanol : acetic acid : water (4 : 1 : 1). The upper band is the yellow component; the next below it, the gray component. Band at lowest Rf is RL 0.25 of table 1.

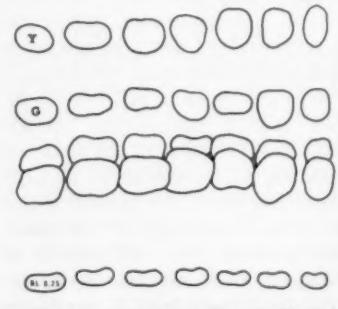

FIG. 7. Chromatograms *B. woodhousei* × *B. speciosus* hybrids developed in *n*-butanol : acetic acid : water (4 : 1 : 1).

TABLE 1. *Components of parotoid glands of* Bufo *species and their hybrids. Composite representation of patterns referred to in text. A dash indicates no data available*

		Y[1]	Y[2]	RL (0.25)	Cream no. 1	Cream no. 2	Gray	RL 1[1] (0.45 in 4 : 1 : 1)	RL 2 (lowest in 1 : 1 : 1)	Sex	Collection site
B. woodhousei	1	X	0	X	X	0	0	X	X	♂	Plano, Texas
	2	0	0	X	X	X	?[2]	X	X	♂	Plano, Texas
	3	?	0	0	?	0	0	X	?	♂	Plano, Texas
	4	X	0	X	X	X	?[2]	X	X	♀	Houston, Texas
	5	?	0	0	X	?	0	X	X	♀	Waller Co., Texas
	6	X	0	X	X	0	0	X	X	♂	Waller Co., Texas
	7	?	0	X	X	X	0	X	?	♂	Waller Co., Texas
	8	?	0	0	?	?	0	X	?	♀	Waller Co., Texas
	9	?	0	0	X	X	0	X	?	♀	Waller Co., Texas
B. speciosus	1	?	0	X	X	0	X	X	X	♂	Austin, Texas
	2	X	0	X	0	0	?	X	X	♀	Austin, Texas
	3	?	0	X	X	0	X	X	0	♂	Austin, Texas
	4	?	0	X	X	0	X	X	X	♂	Austin, Texas
	5	?	0	X	0	0	?	X	0	♂	Austin, Texas
	6	X	0	X	X	0	X	X	X	♂	Utley, Texas
	7	0	0	0	0	0	0	X	0	♂	Utley, Texas
	8	X	0	X	X	0	X	X	X	♀	Sinton, Texas
	9	0	0	0	0	0	0	X	0	♀	Sinton, Texas
B. cognatus	1	0	X	?	X	0	X	X	0	♂	Arizona
	2	0	X	X	X	0	X	X	0	♂	Arizona
	3	0	X	X	?	0	X	X	0	♂	Arizona
	4	0	X	X	?	0	X	X	0	♂	Arizona
	5	0	X	X	?	0	X	X	0	♂	Arizona
	6	0	X	?	–	–	?	X	0	♀	Arizona
	7	0	X	–	–	–	–	X	0	♀	Arizona
	8	0	X	–	–	–	–	X	0	♀	Arizona
	9	0	X	–	–	–	–	X	0	♂	New Mexico
woodhousei × *speciosus*	1	X	0	X	X	0	X	X	X	♂	
	2	X	0	X	X	0	X	X	X	♂	
	3	X	0	X	X	0	X	X	X	♀	
	4	X	0	X	X	0	X	X	X	♂	
	5	X	0	X	X	?	X	X	X	♀	
	6	X	0	–	X	0	X	X	–	♀	
	7	X	0	–	X	0	X	X	–	♀	
woodhousei × *cognatus*	1	?	X	X	X	0	0	X	0	♂	
	2	?	X	X	X	0	0	X	0	♀	
	3	–	–	X	–	–	–	X	0	♀	

[1] Reddish-lavender spot.

[2] Not actually typical gray color and very faint. Y^1 = upper yellow band. Y^2 = lower yellow band.

(as in the specific case cited), or tailing prevented a valid judgment.

Inspection of the chromatograms clearly allowed the conclusion that the variations of patterns among the individuals of the same species are not obviously correlated with the geographical origin or the sex of the frog. In fact, no chromatographic differences between the sexes were observed.

DISCUSSION

The results of the current investigation establish the fact that parotoid gland secretions of *Bufo* species provide a source of individual biochemical variation capable of being analyzed by chromatographic methods. The differences are both quantitative and qualitative and involve two dis-

tinct classes of chemical compounds and probably others undisclosed by the methods used. In general, it appears that species which bear close resemblance and which may be interbred show the more similar biochemical patterns in their parotoid secretions. Data on this point are inadequate to support a categorical statement, but the phenolic patterns of *Bufo marinus*, for example, were quite unlike those representative of *woodhousei*, *cognatus*, and *speciosus*.

The fundamental question concerning chromatographic differences is whether or not they reflect genetic differences. Because the original parents of the hybrid toads were not available, the strong circumstantial evidence that otherwise would have been acquired from the F_1 generation is lacking. The data reported here do not permit a conclusion, even though the toads were kept under more or less standard conditions. However, in all cases where a second extraction of the same toad was carried out, the chromatographic patterns were alike. There is reason to expect, on the basis of biochemical genetic studies of a variety of organisms, that the substances under consideration are likely to be under genetic control. If one assumes, for the sake of the argument, that all differences observed are real genetic differences, then they provide an excellent opportunity for a number of different studies oriented toward population and biochemical genetics, phylogeny, etc. If, as appears somewhat unlikely, the differences are entirely nongenetic, they still must be accounted for. Since, presumably the constituents of the glands accumulate over a period of days or weeks, and the phenolic substances more than likely represent end products of an essentially irreversible metabolic pathway, it is not likely that circumstances of the immediate past dictate the composition of the secretion. Similarly, any event during an extended period of time could affect the composition of the glandular secretion, e.g., some situation which resulted in physiological stress might temporarily modify the pattern of glandular secretion. If this resulted in the formation of a "new" substance that could not be metabolized or removed, it would be disclosed as a modified chromatographic pattern. Obviously, one should not jump to a hasty conclusion and much more work is needed.

It is interesting that no obvious differences of chromatographic pattern are associated with sex in the toads. In other work, particularly with *Drosophila*, great differences in the chromatographic patterns of males and females were found (Buzzati-Traverso, 1953; Fox, 1956). This was particularly evident in Fox's work because he had isogenic material, and of 19 different fluorescent substances only 10 were common to both males and females and nine of these showed quantitative differences between the sexes.

After comparing chromatograms of *B. woodhousei*, *B. cognatus*, and *B. speciosus* the conclusion is that they are generally rather similar. Those differences which are consistent enough to suggest that they involve species—specific characters (e.g., Y^1, Y^2, gray, cream 2, and RL 2 of table 1) are such as not to imply particular affinity between any of the three species. One could not conclude from these results, for example, that *B. woodhousei* should be grouped separately from the other two. Of course, at this stage it is premature to draw conclusions from the chromatographic evidence that bears upon the systematic relationships of the species.

The exact chemical natures of the substances appearing on the chromatograms are unknown, and it is hoped that work along the line of further chemical characterization will proceed in due course. A number of constituents of toad parotoid gland secretions have been identified, including sterols, sterol glycosides, nitrogenous derivatives of sterols, and the previously mentioned bufotenines. Principal interest in these compounds has arisen from their pharmacological properties, involving mostly cardiac effects.

In a number of studies of hybrids, the parents of which differ in one or more bio-

chemical properties, the hybrids contained the components of both parents (e.g., Pryor and Bryant, 1958). This tendency has also been described in serological investigations of hybrids (Irwin, 1951; Hall, 1959). Theoretically, for complex heterozygotes, this would not be expected to be inevitable. There is a tendency for all *Bufo* hybrids to contain the substances which occur sporadically among the parental species. Note, for example, the distribution of "gray." Also cream 1 was present in larger quantity in all hybrids than in most of the parental types. In contrast, spot RL 2 was absent from the three *B. woodhousei* × *B. cognatus* hybrids although present in all of nine *B. woodhousei* individuals. It is possible, of course, that the actual *B. woodhousei* parent was exceptional and lacked RL 2.

The results of the work reported here indicate to us that extension of these techniques, including a comprehensive genetic analysis, would be fruitful.

Summary

Alcoholic extracts of parotoid gland secretions of several *Bufo* species were chromatographed in two solvents and sprayed, separately, with ninhydrin and a general phenol detecting reagent. Among the species examined, *B. cognatus*, *B. speciosus*, and *B. woodhousei*, together with certain of their hybrids, were of particular interest. Amino acid patterns of different species differed, but there was also a surprisingly high degree of intraspecific variation. However, species differences in phenolic constituents did appear, and though intraspecific differences were apparent in this case too, there were a number of consistent differences. The hybrids were notably more uniform in their chromatographic patterns, and in general appeared to contain the substances associated with both parental species. From the data acquired, one could not conclude that any two of the three species cited were particularly closely related. Although it has not yet been established that these distinct biochemical differences are genetically controlled, the toads subsisted on standard diets, and it seems highly probable that genetic differences are reflected in the various patterns. Further studies along these lines may be expected to be applicable to problems of phylogeny, biochemical, and population genetics of *Bufo*.

Literature Cited

Alston, R. E., and H. E. Irwin. 1961. The comparative extent of variation of free amino acids and certain "secondary" substances among *Cassia* species. Am. J. Bot., **48**: 35–39.

Blair, W. F. 1956. Call difference and isolation mechanisms in southwestern toads (genus *Bufo*). Texas J. Sci., **8**: 87–106.

——. 1959. Genetic compatibility and species groups in U. S. toads (*Bufo*). Texas J. Sci., **11**: 427–453.

Buzzati-Traverso, A. A. 1953. Paper chromatographic patterns of genetically different tissues: a contribution to the biochemical study of individuality. Proc. Nat. Acad. Sci., **39**: 376–391.

——. 1960. Paper chromatography in relation to genetics and taxonomy. *In*: New Approaches in Cell Biology, pp. 95–123.

Deulofeu, V. 1948. The chemistry of the constituents of toad venoms. Fortschr. Chem. org. Naturstoffe, **5**: 241–266.

Fox, A. S. 1956. Chromatographic differences between males and females in *Drosophila melanogaster* and role of X and Y chromosomes. Physiol. Zool., **29**: 288–298.

Hall, O. 1959. Immuno-electrophoretic analyses of allopolyploid ryewheat and its parental species. Hereditas, **45**: 495–504.

Irwin, M. R. 1951. Genetics and immunology. *In*: Genetics in the 20th Century. L. C. Dunn, ed. Macmillan, New York.

Kirk, R. L., A. R. Main, and F. G. Beyer. 1954. The use of paper partition chromatography for taxonomic studies of land snails. Biochem. J., **57**: 440–442.

Possingham, J. V. 1956. The effect of mineral nutrition on the content of free amino acids and amides in tomato plants. I. Aust. J. Biol. Sci., **9**: 539–551.

Pryor, L. D., and L. H. Bryant. 1958. Inheritance of oil characters in *Eucalyptus*. Proc. Linnaean Soc. New South Wales, **83**: 5,564.

Turner, B. L., and R. E. Alston. 1959. Segregation and recombination of chemical constituents in a hybrid swarm of *Baptisia laevicaules* × *B. viridis* and their taxonomic implications. Am. J. Bot., **46**: 678–686.

NATURAL SELECTION BETWEEN TWO SPECIES OF TEPHRITID FRUIT FLY OF THE GENUS *DACUS*

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Over 30 species of tephritid (trypetid) fruit flies of the subfamily Dacinae are endemic to Australia (Hardy, 1951). They lay their eggs into a variety of native fruits. Since the planting of cultivated fruits a few species have spread to these hosts. By far the most common species in cultivated fruits is *Dacus tryoni tryoni*, a species which is morphologically very similar to *D. dorsalis* of the Orient and Hawaii.

In the course of studying geographic variation in *D. tryoni tryoni*, Bateman (1958) found that when he reared flies from guavas collected in the temperate regions of Australia, such, for example, as the Sydney district, all the flies which emerged were *D. tryoni tryoni*. But in the tropical parts of Australia a second fly, which Hardy (1951) considered to be a variant of *D. tryoni* which he called *D. tryoni neohumeralis*, occurred together with *D. tryoni tryoni* in flies bred from guavas. The fly which Hardy (1951) called *neohumeralis* is morphologically similar to *tryoni* except for its brown humeral cali. In *tryoni* these are bright yellow. The difference is quite conspicuous to the naked eye. Where the two occur sympatrically, intermediate color forms are also found but constitute quite a small percentage of the population. Bateman (1958) showed that they are hybrids of *tryoni* and *neohumeralis*. Table 1 shows the tendency for *tryoni* to be found in greater proportion in guavas from the tropics (Cairns) as compared with temperate regions (Sydney). Intermediate proportions occur in Brisbane, which is 500 miles north of Sydney and 1,500 miles south of Cairns. The proportion of the two was different in other fruits, but in general *neohumeralis* becomes less common as one proceeds south from the tropics. It is absent from Sydney and places south of Sydney.

Bateman (1958) found that *tryoni* males mated with *neohumeralis* females in the laboratory, producing fertile hybrids which he called type 1 intermediates. The reciprocal cross did not take place; the females of *tryoni* just did not mate with males of *neohumeralis*. The F_1 hybrids of the former cross produced in the F_2, *tryoni*, *neohumeralis* type 1 intermediates and a second intermediate form which Bateman called type 2 intermediate. Type 2 forms did not mate with *tryoni*. From preliminary studies Bateman concluded that at least two pairs of genes were involved in the differences between *tryoni* and *neohumeralis*.

The existence of intermediates in natural populations (table 1) indicates that some hybridization takes place in nature. But the low frequency of intermediates suggests a high degree of sexual isolation between *tryoni* and *neohumeralis*. They should be regarded as sibling species. I shall therefore refer to them as two species *tryoni* and *neohumeralis*.

The purpose of the present study was to investigate factors that could be responsible for the increase in relative abundance of *neohumeralis* from south to north as compared with *tryoni*. The hypothesis I set out to test was that *neohumeralis* was better adapted than *tryoni* to survive and multiply in tropical climates and that the reverse was true in temperate climates. The one component of climate studied was temperate.

Since the two species occur sympatrically in the field, and I knew that they were able to interbreed in the laboratory, I considered it desirable to compare them when living together in population cages where they had opportunity to interbreed, though

TABLE 1. *Per cent of* D. tryoni tryoni *and* D. tryoni neohumeralis *bred from guavas* (Psidium guajava) *collected in the tropics (Cairns), sub-tropics (Brisbane), and temperate region (Sydney) of Australia*

Date collected	Locality	Per cent			Total no. of flies
		tryoni	*neohumeralis*	intermediates (hybrids)	
March 1956[1]	Cairns	9.3	90.7	not recorded	1,546
	Brisbane	93.3	6.7	not recorded	1,779
	Sydney	100	0	0	many thousands
February 1957[2]	Cairns	5.7	91.4	2.9	1,535
	Brisbane	—	—	—	—
	Sydney	100	0	0	many thousands
March 1960[2]	Cairns	40.0	49.0	11.0	173
	Brisbane	60.8	35.0	4.2	1,539
	Sydney	100	0	0	many thousands

[1] Collected by M. A. Bateman.
[2] Collected by L. C. Birch.

a population cage presumably gives the flies more opportunity to interbreed than they would have in nature. It would, of course, be possible to compare the two species as separate populations, but this was not done.

PLAN OF EXPERIMENTS

A factorial experiment was designed in which *tryoni* and *neohumeralis* were bred together for successive generations in a special type of population cage. The initial population consisted of 80% *tryoni* and 20% *neohumeralis* in some experiments, and 20% *neohumeralis* and 80% *tryoni* in others. The proportions of the two species and their recognizable hybrids (type 1 and type 2 intermediates) were estimated at monthly intervals. From this information it was anticipated that conclusions could be drawn about the relative superiority in survival and/or reproduction of one species compared with the other. Experiments were done at two temperatures, 22° C ± 0.5° C and 25° C ± 0.5° C, at a relative humidity of 70% ± 5%, and at two densities "crowded" and "uncrowded." The reason for varying the density was to find out if superiority depended upon density. The way this was done is described later on.

Dacus lays its eggs into sound fruit where the larvae develop until maturity when they leave the fruit to pupate in the soil. The sources of food for adults in nature are not yet completely known. In the laboratory, sucrose, protein hydrolysate of yeast and water constitute a suitable diet for adults. Adults were kept and fed in cages separate from the larvae. In experiments where flies were "crowded," 500 flies (half males and half females) one to two weeks old were introduced into a nylon cage 18″ × 13″ × 13″ and provided with food and water. The quantity of food was kept constant by regular renewal. Oviposition sites were provided twice a week by introducing into the cage hollow domes of apple skin fixed with wax onto pieces of glass. The discs of apple skin were kept in the cage for 7 hours. The eggs were removed with a brush to moistened filter paper. On hatching at the temperatures of the experiment they were transferred to a plastic dish containing 500 ml of larval food. This was then enclosed in a nylon cage. Thus one day's collection of eggs were all put onto one dish of medium. The medium consisted of dried carrot which had been finely ground, mixed with water, and brought to a pH of 4.5 with

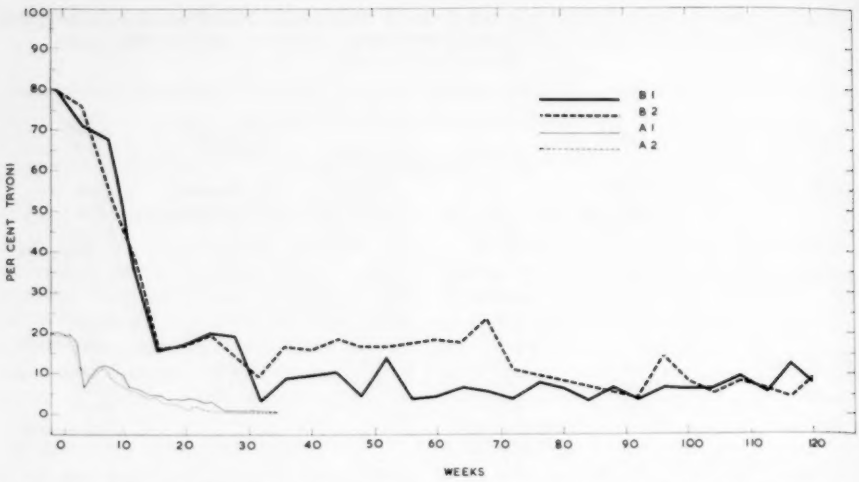

FIG. 1. Change in proportion of "*tryoni*" when crowded at 25° C. A_1 and A_2 are replicates in which the initial proportion of *tryoni* was 20% and the initial proportion of *neohumeralis* was 80%. B_1 and B_2 are replicates in which the initial proportion of *tryoni* was 80% and the initial proportion of *neohumeralis* was 20%.

hydrochloric acid. It contained 2 per cent dried brewers yeast and .05 per cent of the fungicide "Nipagin." These procedures meant that the quantity and quality of larval food were kept constant. The mature larvae hop out of the dish into moist sawdust on the bottom of the cage, where they pupate. All pupae were placed in the cage of adults, where they subsequently emerged. The arrangement is analagous to a *Drosophila* population cage except that adults and immature stages are kept separate and eggs are collected from adults twice a week instead of all the time.

At monthly intervals 500 adults were temporarily removed from the cage, anaesthetized with carbon dioxide, and identified under a binocular microscope. They were sorted into *tryoni*, *neohumeralis*, type 1 and type 2 intermediates. In one experiment the total number of adults was determined at weekly intervals. Instead of letting adults emerge from pupae in the population cage, they emerged in separate cages. They were counted before being introduced into the population cage. The flies which had died in the population cage were collected and counted. The difference each week gave the increase or decrease in number of flies week by week. The procedure was very laborious, since there were usually about 10,000 flies in the population cages when flies were "crowded."

In experiments in which flies were kept "uncrowded" the total number of adults was kept at 500 all the time and the total number of larvae per 500 ml of medium was 1,000. The procedure was as follows. After 500 flies were introduced into the cage, eggs were collected twice a week for three weeks and these eggs were placed on dishes of medium, 1,000 eggs per dish. When the adults emerged from each week's collection the frequency of the different types was estimated on a sample of 500 flies. A sample of 500 adults was then made up, being constituted of the mean frequency of the different types for the three-week period. This was the adult population for the next generation. The process was repeated "generation" after "generation," each "generation" taking about 8 weeks to complete.

All experiments were conducted in con-

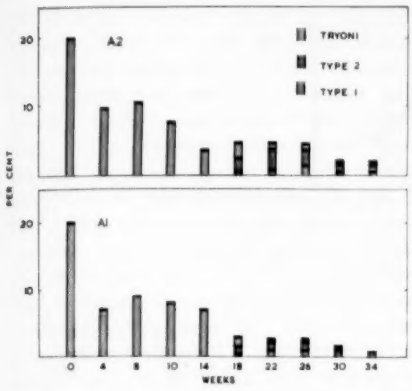

FIG. 2. Proportions of "*tryoni*," "*neohumeralis*," and intermediates (type 1 and type 2) in replicate populations A_1 and A_2 when *tryoni* and *neohumeralis* were crowded at 25° C with an initial proportion of 20% *tryoni* and 80% *neohumeralis*. The proportion of "*neohumeralis*" is the difference between the top of the columns and 100%.

stant-temperature rooms in which the illuminance was rigidly controlled. This was necessary since Barton-Browne (1956, 1957) has shown that frequency of mating and the fecundity of *tryoni* is a function of illuminance. Fluorescent lights were automatically turned on at a high illuminance for 11½ hours of the day; this was followed by half an hour of "artificial dusk" of about one foot-candle illuminance for mating. This was followed by 12 hours of darkness.

NATURAL SELECTION OF *TRYONI* AND *NEOHUMERALIS* WHEN "CROWDED" AT 25° C

The change in proportion of *tryoni* when crowded with *neohumeralis* at 25° C is shown in fig. 1. The two lower curves (A1, A2) show the change in proportion of *tryoni* in two replicate experiments when the initial population consisted of 100 *tryoni* (i.e. 20%) and 400 *neohumeralis* (80%). Two two upper curves (B1, B2) show the change in proportion of *tryoni* when it initially constituted 80% of the population. When the initial proportion was 20% *tryoni* (A) the proportion of *tryoni* dropped rapidly and by the 34–5th week it was zero. A mean 99% of the population were then *neohumeralis*, 0.5% were type 1 intermediates, and 0.5% were type 2 intermediates. The detailed constitution of the populations during their history is shown in the column diagrams of fig. 2. When the initial population consisted of 80% *tryoni* (B1, B2), the proportion of *tryoni* again dropped rapidly for the first 15 weeks but instead of becoming zero it fluctuated around 10% for the remaining two years of the experiment. The two replicates were remarkably similar. A striking difference was the appearance of hybrids in the 8th week in B1 and B2, compared with the 18th week in A1 and A2, and the

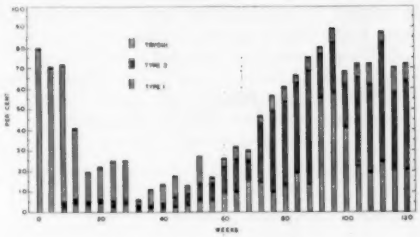

FIG. 3. Proportions of "*tryoni*," "*neohumeralis*," and intermediates (type 1 and type 2) in population B_1 when *tryoni* and *neohumeralis* were crowded at 25° C with an initial proportion of 80% *tryoni* and 20% *neohumeralis*. The proportion of "*neohumeralis*" is the difference between the top of the columns and 100%.

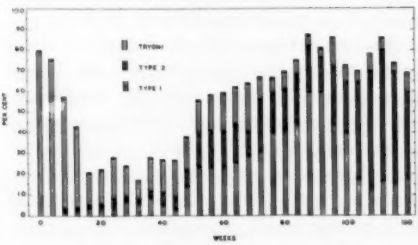

FIG. 4. Proportions of "*tryoni*," "*neohumeralis*," and intermediates (type 1 and type 2) in population B_2 (replicate of B_1) when *tryoni* and *neohumeralis* were crowded at 25° C with an initial proportion of 80% *tryoni* and 20% *neohumeralis*. The proportion of "*neohumeralis*" is the difference between the top of the columns and 100%.

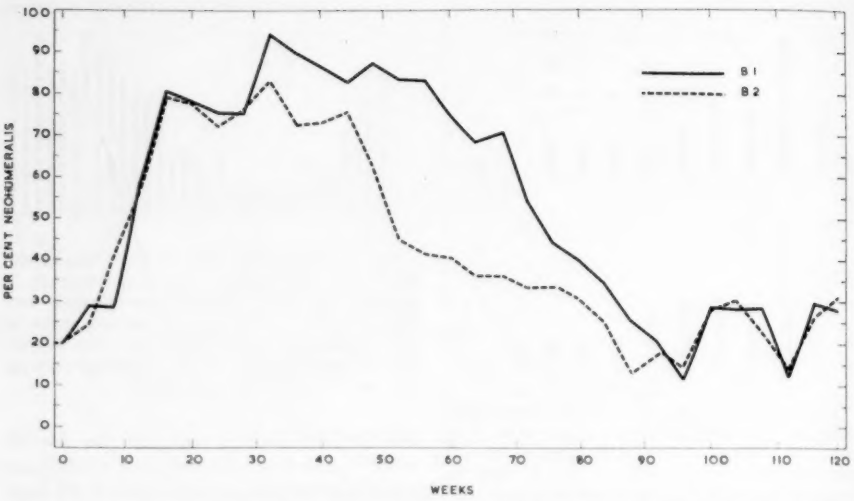

FIG. 5. Change in proportion of "*neohumeralis*" when crowded at 25° C. B_1 and B_2 are replicates in which the initial proportion of *tryoni* was 80% and the initial proportion of *neohumeralis* was 20%.

frequency of hybrids increased gradually (figs. 3 and 4) with corresponding decrease in proportion of *neohumeralis* (fig. 5). Thus by the 100th week in one replicate the percentage of hybrids was 64.8 and in the other replicate it was 55.8. The four figures (1, 3, 4, 5) can be summarized by saying that for the first 30 weeks selection was against *tryoni* and in favor of *neohumeralis*, but with the establishing of a population of hybrids, selection was then against *neohumeralis* until it constituted about 20% of the population.

The persistence of both *tryoni* and *neohumeralis* in the populations with initial proportion 80% *tryoni* and 20% *neohumeralis* was associated with the existence of a large proportion of hybrids. The extinction of *tryoni* in populations which initially contained only 20% *tryoni* was associated with a very small population of intermediates. There were fewer *tryoni* to start with, selection was against *tryoni*, their females did not accept *neohumeralis* males; these three things evidently prevented *tryoni* genes from becoming incorporated into the population to any significant extent.

A number of possible mechanisms could explain the coexistence of the four identifiable color types in balance in these experiments in which the initial population consisted of 80% *tryoni* and 20% *neohumeralis*. Unfortunately it is not possible to analyze this at all fully since colors do not identify genotypes. A fly called *tryoni* at the beginning of the experiment is different genetically from flies identified as *tryoni* later on in the experiment. The same applies to other color types. This is shown in mating experiments reported below. I shall therefore refer to flies in the hybridizing populations as "*tryoni*" and "*neohumeralis*" to distinguish them from *tryoni* and *neohumeralis* present at the beginning of the experiments.

Since color is not a marker for a particular genotype the data are inadequate to determine if heterosis of genes is responsible for the coexistence of "*tryoni*," "*neohumeralis*," and the two intermediates. The only evidence obtained which is adequate to provide at least a partial explanation concerns change in the mating pattern during the course of the experiments.

Change in mating pattern.—*Tryoni* and *neohumeralis* do not mate at random. In

the field up to about 10% of the flies are hybrids. When brought into the laboratory *tryoni* females do not mate with *neohumeralis* males, and type 2 males and females do not mate with *tryoni* (Bateman, 1958). However, after several generations together in the laboratory the proportion of hybrids increases. In figs. 3 and 4 they dominate the population. This suggests that the sexual isolation which existed initially in these populations had broken down. The results of crosses shown in table 2 substantiate this. Table 2 shows the percentage of females inseminated in the fourth week of adult life. For each cross two lots of 30 pairs of flies were kept with food in perspex cages at 25° C before females were dissected to see if spermathecae contained sperm or not. Crosses A, B, C, and D were made with flies from stocks of *tryoni* and *neohumeralis* kept separately for three generations in the laboratory. Type 2 males and females in crosses E and F were obtained from the progeny of type 1 flies, which in turn were obtained by crossing *tryoni* males and *neohumeralis* females from stocks kept separately. The results of these six crosses confirm what Bateman (1958) had previously found, namely that *tryoni* females did not mate with *neohumeralis* males and type 2 did not mate with *tryoni*. A small percentage of *neohumeralis* females mated with *tryoni* males. Crosses G, H, I, J were done with unmated flies obtained from the interbreeding population of all types illustrated in figs. 1, 3, 4, and 5. The flies were taken out of the cages in the 150th week of the experiment, that is after all four types had coexisted for three years together. The significant features are that a high proportion of "*neohumeralis*" females from this source was inseminated by "*tryoni*" males (compare G with C), some "*tryoni*" females were inseminated by "*neohumeralis*" males (compare H with D), a high proportion of type 2 mated with "*tryoni*" (compare I and J with E and F). In each cross, fertile eggs were laid. The fertility was 80% for G, 33% for H, and 50% for I and J. The conclusion to be drawn is that sexual isolation had broken down in the interbreeding population. The remaining crosses K to P confirm that "*tryoni*" and "*neohumeralis*" in the interbreeding population were different from *tryoni* and *neohumeralis* in the original stocks. In these crosses flies from stocks were mated with flies from the interbreeding population. The percentage insemination of females was much the same as if all the flies had come from the interbreeding population (contrast K–P with A–F). None of the relevant comparisons are significantly different. The flies from the interbreeding population evidently mate with anything from the stocks with one exception: in cross O stock *tryoni* accepted type 2 from the interbreeding population but not to anything like the extent to which "*tryoni*" females from the interbreeding population accepted type 2 from the same population. The result of cross M may be different

TABLE 2. *Per cent insemination of females in crosses of stock flies compared with those from interbreeding populations of* "tryoni" *and* "neohumeralis" *and in crosses involving both these sources. Stock flies are signified by subscript S, those from the interbreeding population are signified by subscript I*

	Cross		Per cent females inseminated	Number of females
	Male	Female		
A	T_S	T_S	31	50
B	N_S	N_S	37	50
C	T_S	N_S	5	25
D	N_S	T_S	0	25
E	II_S	T_S	0	50
F	T_S	II_S	0	50
G	T_I	N_I	78	30
H	N_I	T_I	13	30
I	II_I	T_I	78	30
J	T_I	II_I	41	25
K	T_S	N_I	56	40
L	N_I	T_S	14	40
M	T_I	N_S	0	30
N	N_S	T_I	20	30
O	II_I	T_S	13	35
P	T_S	II_I	41	35

T = *tryoni*.
N = *neohumeralis*.
II = type 2 intermediates.

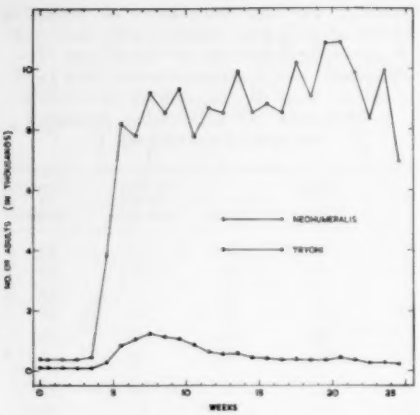

FIG. 6. Total numbers of "*tryoni*" and "*neohumeralis*" for the first 25 weeks in population A_1 when crowded at 25° C. The initial proportions consisted of 20% *tryoni* and 80% *neohumeralis*.

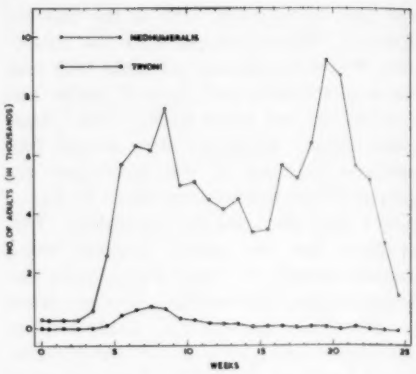

FIG. 7. Total numbers of "*tryoni*" and "*neohumeralis*" for the first 25 weeks in population A_2 (replicate of A_1) when crowded at 25° C. The initial proportions consisted of 20% *tryoni* and 80% *neohumeralis*.

from the result of cross C, but the numbers are too small to be significant. The overall conclusion is that after 150 weeks of interbreeding the four types of identifiable flies were no longer the same as the stocks from which they originated. The difference identified was in their pattern of mating.

This result leads naturally to the further questions—what is the mating pattern in the interbreeding population, and can it help to explain the coexistence of all four color types? Four cages of unmated flies from the interbreeding population in its 150th week were set up as follows: 10 of each of the four types of females were caged with (a) 40 "*tryoni*" males, (b) 40 "*neohumeralis*" males, (c) 40 type 1 males, (d) 40 type 2 males. After three weeks the females were dissected. The results are not tabulated since the percentage insemination of the four different types of females was not significantly different. A mean of 75% were inseminated. The conclusion is that each sort of male mated with each sort of female. This result is inconclusive without further knowledge on the frequency of the different sorts of mating, which was impossible to obtain without very much more elaborate experiments.

THE TREND IN NUMBERS OF *TRYONI* AND *NEOHUMERALIS* WHEN CROWDED AT 25° C

The actual numbers of *tryoni* and *neohumeralis* were counted weekly for the first 25 weeks in the experiment (A1, A2, fig. 1), in which the initial proportion of *tryoni* was 20%. The reason for doing this was that superiority in survival and/or reproduction might depend upon density, so information on change in density might have interpretive value. Furthermore, little is known about trends in numbers of flies in population cages.

Figs. 6 and 7 show that in one replicate the initial rise of "*neohumeralis*" was followed by relatively small fluctuations in numbers around 9,000 to 10,000 adult flies, while in the second replicate the initial rise in numbers of "*neohumeralis*" was followed by a severe fluctuation without any suggestion of an asymptote. The latter replicate resembles Nicholson's (1957) experiment with the blowfly *Lucilia cuprina* when he supplied either (a) ample food for adults but restricted quantities of food for larvae, in which case larvae died in enormous numbers causing severe fluctuations in numbers, or (b) ample larval food but restricted quantities of food for adults, in which case adults died in enor-

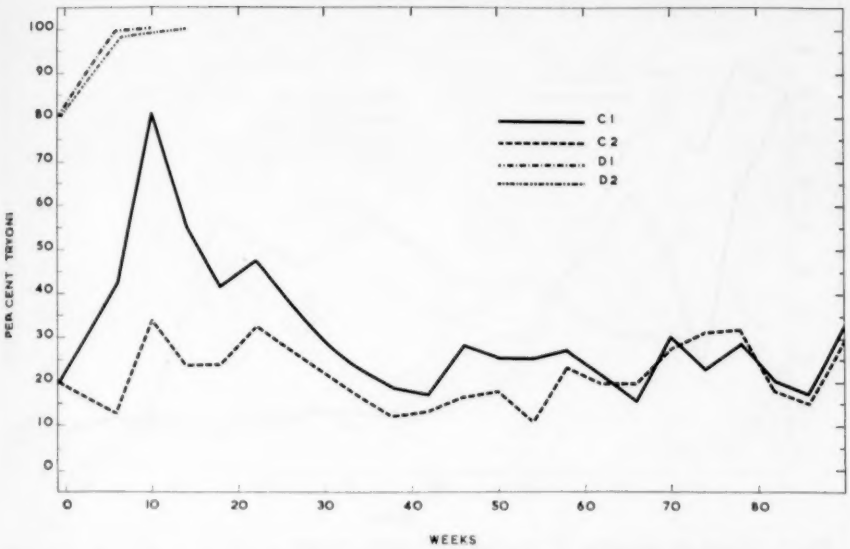

FIG. 8. Change in proportion of "*tryoni*" when crowded at 22° C. D_1 and D_2 are replicates in which the initial proportion of *tryoni* was 80% and the initial proportion of *neohumeralis* was 20%. C_1 and C_2 are replicates in which the initial proportion of *tryoni* was 20% and the initial proportion of *neohumeralis* was 80%. The combined percentage of *tryoni* in this figure and *neohumeralis* in fig. 9 do not total 100% since intermediates increase rapidly after 10 weeks.

mous numbers causing severe fluctuations in numbers. The other replicate (fig. 6) in which there were no violent fluctuations resembles Nicholson's experiment in which both adult and larval food were supplied daily in constant limited quantities such that the effects of larval deaths and adult deaths opposed each other. The violence of the oscillations was in this case greatly reduced. Nicholson's experiments showed that relatively small changes in quantities of adult and larval food can be responsible for changing a fluctuating population into a relatively steady one. Such differences could apply to the two replicates of *Dacus* (figs. 6 and 7) despite the fact that efforts were made to keep the quantity of food the same in each replicate.

NATURAL SELECTION OF *TRYONI* AND *NEOHUMERALIS* WHEN CROWDED AT 22° C

The two curves (D1, D2) in the top left corner of fig. 8 and in the bottom left corner of fig. 9 show what happened at 22° C when the initial proportion of *tryoni* was 80%. "*Tryoni*" rapidly increased in proportion and "*neohumeralis*" decreased until by the 13th week it was extinct. The result is the opposite of what happened at 25° C, where "*neohumeralis*" was favored and "*tryoni*" became extinct. Very few intermediates were formed. None was recorded until the 9th week when 0.1% were type 2. By the 13th week there were still only 0.1% type 2.

The lower two curves in fig. 8 (C1, C2) show what happened to the proportion of *tryoni* when initially it constituted 20% of the population. In one replicate it increased to 80% within the first 10 weeks, and in the other to 30%, indicating again strong selection against "*neohumeralis*." However, after the 10th week "*tryoni*" fell in proportion. This coincided with an increase in the proportion of intermediates (figs. 10 and 11). "*Neohumeralis*" contin-

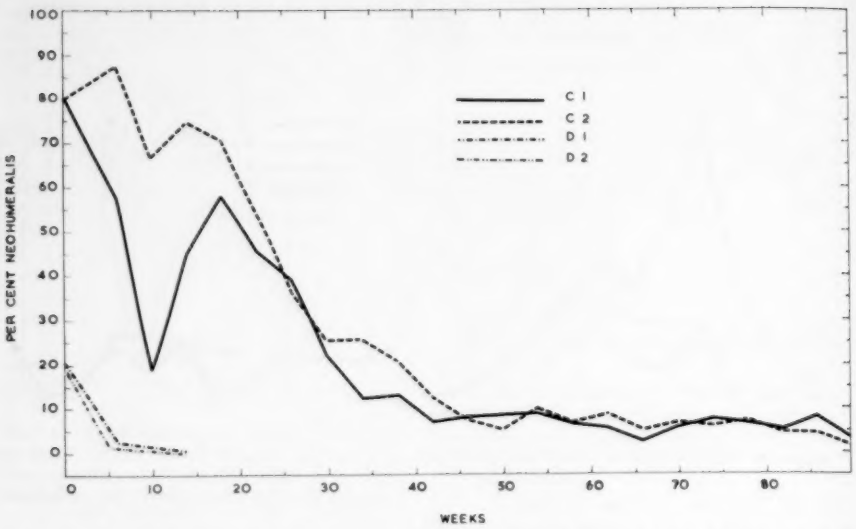

FIG. 9. Change in proportion of "*neohumeralis*" when crowded at 22° C. C_1, C_2, D_1, D_2 as in fig. 8.

ued to fall (fig. 9) until it reached an equilibrium at about the 42–5th week. At this stage the constitution of the population was:

	"tryoni"	*"neohumeralis"*	*type 1*	*type 2*
Replicate C1	17.2%	6.8%	41.0%	35.0%
Replicate C2	13.4%	12.2%	40.0%	34.4%

When classified into these four color classes the majority were of type 1 and type 2. The detailed changes in proportion of "*neohumeralis*" and the intermediates are shown in figs. 10 and 11. There are some striking parallels in the results of this experiment conducted at 22° C and that conducted at 25° C. At 25° C selection

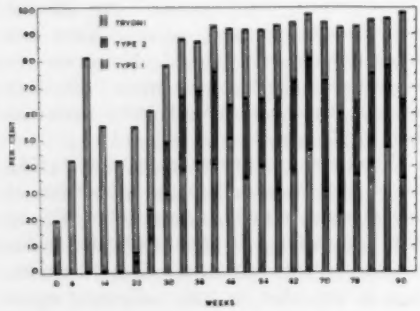

FIG. 10. Proportion of "*tryoni*," "*neohumeralis*," and intermediates (type 1 and type 2) in population C_1 (crowded at 22° C with initial proportion of 20% *tryoni* and 80% *neohumeralis*). The proportion of "*neohumeralis*" is the difference between the top of the columns and 100%.

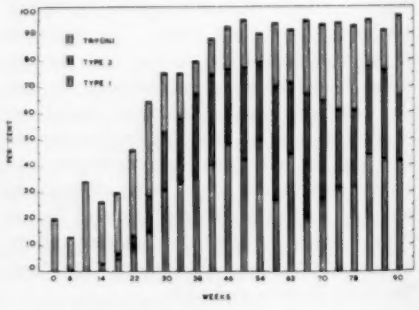

FIG. 11. Proportions of "*tryoni*," "*neohumeralis*," and intermediates (type 1 and type 2) in population C_2 (crowded at 22° C with initial proportion of 20% *tryoni* and 80% *neohumeralis*). The proportion of "*neohumeralis*" is the difference between the top of the column and 100%.

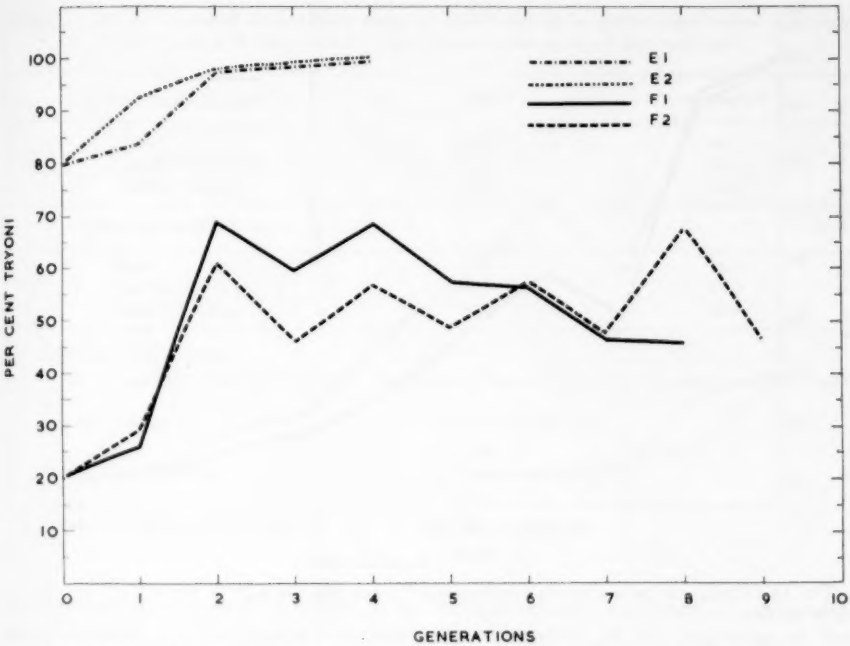

FIG. 12. Change in proportion of "*tryoni*" when "uncrowded" at 25° C. E_1 and E_2 are replicated populations with initial proportions of 80% *tryoni* and 20% *neohumeralis*. F_1 and F_2 are replicated populations with initial proportions of 20% *tryoni* and 80% *neohumeralis*.

was initially against "*tryoni*." At 22° C it was initially against "*neohumeralis*." A difference in temperature of three degrees reversed the order of superiority of the two species. When the unfavored species constituted a low proportion of the initial population it became extinct in both experiments. When the unfavored species constituted the greater part of the initial population it did not become extinct but persisted in the population. An equilibrium was established of the four color types. At both 22° C and 25° C the intermediates eventually made up the greater part of the population. At equilibrium about 70% of the flies were intermediates. Genetically all or nearly all of the flies must then have been hybrids. At 25° C "*tryoni*" constituted about 9% of the population and "*neohumeralis*" about 25%. At 22° C "*tryoni*" constituted about 25% of the population and "*neohumeralis*" about 6%. So, whether at equilibrium or not, "*neohumeralis*" was superior to "*tryoni*" at 25° C, but "*tryoni*" was superior to "*neohumeralis*" at 22° C.

NATURAL SELECTION OF *TRYONI* AND *NEOHUMERALIS* WHEN "UNCROWDED" AT 25° C

In this experiment, which was done at 25° C, the density of adults was kept at 500 per cage and the density of larvae was kept at 1,000 per 500 ml of medium. The results are shown in figs. 12 and 13. It will be recalled that, when "crowded," selection was initially against *tryoni* and when the initial population consisted of 20% *tryoni* it eventually became extinct. The reverse happened when the initial population consisted of 20% *tryoni* but when flies were "uncrowded." By the 9th generation *neohumeralis* was extinct (fig. 13, compare fig. 6). The population then con-

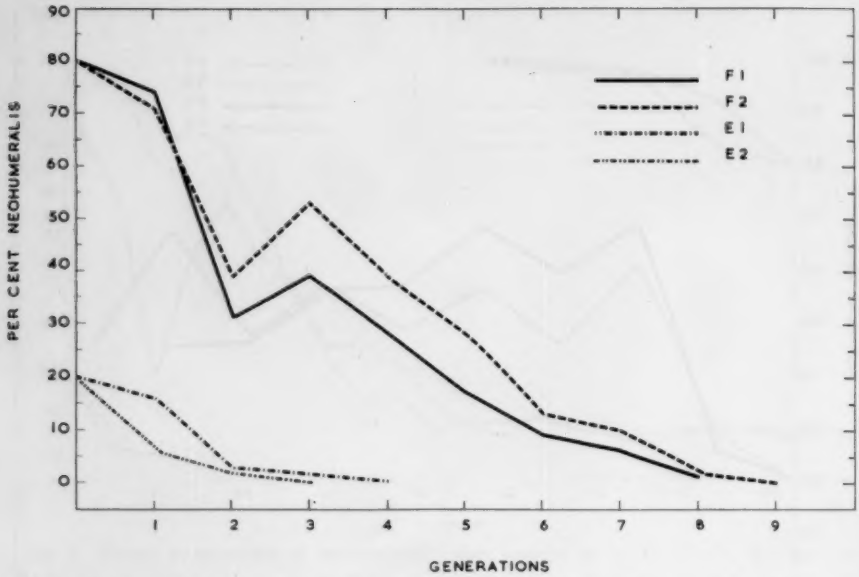

FIG. 13. Change in proportion of "*neohumeralis*" when "uncrowded" at 25° C. E_1, E_2, F_1, F_2 as in fig. 12.

sisted of about half *tryoni* and half intermediates (fig. 14).

When the initial population consisted of 80% *tryoni* the extinction of *neohumeralis* was complete by the 4th generation (E1, E2, fig. 12, compare fig. 1). These results show that whereas *neohumeralis* was superior in crowded populations, in uncrowded populations *tryoni* was superior.

SURVIVAL OF IMMATURE STAGES OF *TRYONI* AND *NEOHUMERALIS* WHEN CROWDED AND UNCROWDED AT 25° C

The reversal of selection in uncrowded as compared with crowded populations invites analysis of the stage or stages in the life history in which selection operates. So an analysis was made of the effect of crowding of larvae on the survival of immature stages. The experiment was factorial with five replicates, two type *tryoni* and *neohumeralis*, two initial densities 1,000 and 2,000 larvae per 500 ml of medium. At each density newly emerged larvae were put on the medium (a) all of one species, (b) both species in equal numbers and mixed together, (c) both species in equal numbers but the two species placed on one-half of the dish each. The point of the variations (b) and (c) was to find out if mixture of two sorts of larvae from the beginning of the experiment had a different effect from their becoming mixed only later on. Altogether 45,000 newly emerged larvae were used in this experiment. The

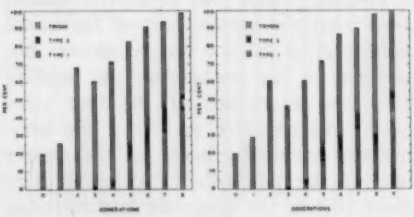

FIG. 14. Proportions of "*tryoni*," "*neohumeralis*," and intermediates (type 1 and type 2) in populations F_1 (on left) and F_2 (on right) when uncrowded at 25° C. The initial proportions were 20% *tryoni* and 80% *neohumeralis*. The proportion of "*neohumeralis*" is the difference between the top of columns and 100%.

TABLE 3. *Per cent survival of immature stages of* tryoni *and* neohumeralis *when reared separately and together at two densities. Each figure is the mean of five replicates*

1,000 larvae per 500 ml medium	*tryoni*	*neohumeralis*
Alone	56.2	44.7
Together unmixed	59.5	46.3
Together mixed	42.8	39.3
2,000 larvae per 500 ml medium		
Alone	47.0	19.8
Together unmixed	53.5	33.2
Together mixed	35.1	15.4

Significant differences:

The figures below give the significant difference at $P = 0.01$ of arcsin $\sqrt{x}$ where x is percentage survival.

"*tryoni*" column	significant difference of arcsin $\sqrt{x}$	$= 11.2$
"*neohumeralis*" column	significant difference of arcsin $\sqrt{x}$	$= 13.1$
tryoni and *neohumeralis* "alone"	significant difference of arcsin $\sqrt{x}$	$= 13.5$

survival to the emergence of the adult is shown in table 3. Three analyses of variance were made: (a) *tryoni* survival (b) *neohumeralis* survival, and (c) on survival of *tryoni* and *neohumeralis* "alone." An estimate of cross column significant differences is possible only in the latter case since the other means are not independent.

When alone, *tryoni* had a significantly greater survival than *neohumeralis* at the higher densities. The survival of *tryoni* was not different when half the animals around it were *neohumeralis*, except at the higher density when its larvae were mixed with *neohumeralis* from the beginning of the experiment. Its survival was then reduced. This indicates that the presence of *neohumeralis* was more deleterious to *tryoni* than an equivalent number of its own kind. This occurred only when larvae were mixed together from the beginning of the experiment. It suggests that the deleterious effect is produced in the early instars.

The survival of *neohumeralis* at the lower density was not significantly different when half the animals around it were *tryoni*. At higher density the same is true when *tryoni* and *neohumeralis* larvae were mixed together at the beginning of the experiment. But when together without being mixed at the early stages the survival of *neohumeralis* was higher when half the animals around it were *tryoni*. This suggests that the later instars of *tryoni* larvae are less deleterious to *neohumeralis* larvae than *neohumeralis* larvae are to themselves.

Significance of differences can be measured in only two of the six cross species comparisons in table 3. However, the data suggest that *tryoni* has higher survival than *neohumeralis* in all comparisons. This result correlates with the superiority of *tryoni* in the "uncrowded" population cages. It throws no light on the superiority of *neohumeralis* in the crowded population cages. This superiority is evidently not associated with larval survival but with adult survival, adult fecundity, or fertility of eggs.

DISCUSSION

These experiments arose out of an attempt to interpret the known distribution and relative abundance of two sibling spe-

cies of tephritid fruit fly in eastern Australia. The two species are sympatric over much of their distributions, for example from tropical Cairns to subtropical Brisbane. But the abundance of *tryoni* in relation to *neohumeralis* is greater in the less tropical part of its distribution, and *tryoni* extends into temperate climatic zones where *neohumeralis* does not exist. Where the species are sympatric, intermediate forms which are hybrids constitute up to about 10% of the natural population in guavas.

The hypothesis I initially set out to test was that *neohumeralis* was better adapted than *tryoni* to survive and multiply in the tropics and that the reverse was true in temperate climates. So populations were reared together in population cages at two temperatures, 25° C and 22° C. Under crowded conditions at 25° C *neohumeralis* was superior to *tryoni*. At 22° C, *tryoni* was superior to *neohumeralis*. This result supports the hypotheses of better adaptation of *neohumeralis* to the warmer climates.

The results of this experiment are similar to those of other studies which have been made. Moore (1952) found that *Drosophila melanogaster* was superior to its sibling species *D. simulans* at 25° C, but that at 15° C the reverse was true. Birch (1953) found that *Calandra oryzae* was superior to *Rhizopertha dominica* at 29° C, but at 31° C the reverse was true. When two sibling species of *Calandra* were compared, one was superior in wheat but the other was superior when the food was maize. Similarly Merrell (1951) showed that differences in quality of food determined whether *Drosophila melanogaster* or *D. funebris* was superior in population cages.

The adaptive differences between *tryoni* and *neohumeralis* present additional complexities. When the two species were reared together under relatively uncrowded conditions at 25° C, and irrespective of whether circumstances permitted the formation of hybrids or not, *tryoni* was superior to *neohumeralis*. This is the reverse of what happened when the flies were crowded. This, so far as I am aware, is the first example of this with two species, though Birch (1955) demonstrated reversal of selective values of two chromosomal types of the one species *Drosophila pseudoobscura* in crowded as compared with uncrowded cultures.

The results of experiments with *tryoni* and *neohumeralis* depended also upon the initial proportions of the two species. When the unfavored species constituted the higher proportion of the initial population hybrids were formed before selection had drastically reduced its numbers. The population then stabilized as a hybrid population. This happened at both 25° C and 22° C. Soon after intermediate color types had appeared in the population, they increased in proportion and then the initially favored species decreased in proportion. Four color types were recognized and were called "*tryoni*," "*neohumeralis*," and type 1 and type 2 intermediates. These established an equilibrium. However most, if not all, of the individuals must have been genetic hybrids at this stage. Mating experiments showed that flies with yellow humeral cali which looked like *tryoni* were not the same as the original *tryoni*, and the same applied to the flies with brown humeral cali which looked like *neohumeralis*. For this reason they are referred to as "*tryoni*" and "*neohumeralis*." Sexual isolation between the original *tryoni* and *neohumeralis* had largely broken down and a population became established which must have consisted entirely, or almost entirely, of genetic hybrids. This result was similar to that obtained by Mettler (1957) with *Drosophila arizonensis* and *D. mojavensis*. These two species hybridize in the laboratory, but they have not been found sympatrically. When reared together in population cages the population came to consist mostly of hybrids and the parental species tended to disappear. Genetic analysis showed that second chromosome heterozygotes and third chromosome heterozygotes exhibited heterosis. This was doubtless

largely responsible for the high frequency of hybrids in the population cages.

Experiments in which *tryoni* and *neohumeralis* were welded into one hybrid population are examples of the reverse of what would have to happen if sexual isolation were to be reinforced by selection. In these experiments selective reinforcement of sexual isolation did not occur, indeed the hybrids were superior to the parental species. These results do not of course demonstrate that selective reinforcement of sexual isolation could not occur under other conditions. I did design another experiment in which I eliminated the intermediate color types as they were formed for each of ten generations, but this did not increase the sexual isolation between *tryoni* and *neohumeralis*. I was not of course eliminating all hybrids, but only those recognized as intermediate color types.

These results pose the interesting question as to how the two species *tryoni* and *neohumeralis* can coexist in nature without much apparent introgression in spite of occasional hybridization, and yet they stabilize as a hybrid population in the laboratory. Nothing is known about this. Presumably the hybrids are very inferior to the parental species in nature in survival and/or in their ability to produce offspring as a result of hybrid matings or matings with the parental species. This is not a case of occasional hybridization at the boundary of two distributions, but occasional hybridization within two broadly overlapping distributions. If selection for sexual isolation can occur, then this should be an ideal situation for it to happen in.

Summary

1. Population cage experiments were maintained for over two and a half years with two sibling species at present known as *Dacus tryoni tryoni* and *D. tryoni neohumeralis*. The latter is the common species in tropical Australia, the former is the commoner one in temperate Australia. The purpose of the experiments was to measure differences in adaptation of the two species to temperature and crowding, with a view to explaining the difference in their distribution and relative abundance in the tropical and temperate regions.

2. When crowded at 25° C, *neohumeralis* was superior to *tryoni*. When crowded at 22° C, *tryoni* was superior to *neohumeralis*. This difference correlates with the greater relative abundance of *neohumeralis* in the tropics. The survival of immature stages of *tryoni* is greater than that of *neohumeralis* in crowded cultures at 25° C. This suggests that the superiority of *neohumeralis* at 25° C was due to a selective advantage in the adult stage.

3. When crowded at 25° C and 22° C, and when the initial proportion of the unfavored species was only 20%, it became extinct in the population.

4. When crowded at 25° C or at 22° C, and when the initial proportion of the unfavored species was 80%, the unfavored species decreased in proportion but did not become extinct. At the same time the proportion of intermediate color types increased until they constituted most of the population. Ultimately the population stabilized as a hybrid population in which sexual isolation had broken down between the two species.

5. The superiority of one species over the other was reversed when populations were kept "uncrowded" at 25° C.

6. The results demonstrate that a difference of 3° C reversed the direction of selection between the two species, as did also the difference between "crowded" and "uncrowded" populations.

Acknowledgments

Miss R. Spurway did much of the technical work involved in these experiments, and she also prepared the graphs. An earlier version of the manuscript was read by Dr. M. A. Bateman, Joint Unit of Animal Ecology in the Department of Zoology, University of Sydney, and Dr. J. M. Rendel, C.S.I.R.O. Division of Animal Genetics, University of Sydney, to whom I am grateful for critical advice.

Literature Cited

Barton-Browne, L. 1956. The effect of light on the fecundity of the Queensland fruit-fly *Strumenta tryoni* (Frogg.). Austral. J. Zool., **4**: 125–45.

——. 1957. The effect of light on the mating behavior of the Queensland fruit fly *Strumeta tryoni* (Frogg.). Austral. J. Zool., **5**: 145–58.

Bateman, M. A. 1958. Ecological adaptations in geographic races of the Queensland fruit-fly, *Dacus* (*Strumeta*) *tryoni*, Froggatt. (Thesis in the Library of the University of Sydney.)

Birch, L. C. 1953. Experimental background to the study of the distribution and abundance of insects. III. The relation between innate capacity for increase and survival of different species of beetles living together on the same food. Evolution, **7**: 136–44.

——. 1955. Selection in *Drosophila pseudoobscura* in relation to crowding. Evolution, **9**: 389–99.

Hardy, D. E. 1951. The Krauss collection of Australian fruit-flies (Tephritidae–Diptera). Pacific Sci., **5**: 115–89.

Mettler, L. E. 1957. Studies on experimental populations of *Drosophila arizonensis* and *Drosophila mojavensis*. Univ. Texas Pub., **9**: 157–81.

Merrell, D. J. 1951. Interspecific competition between *Drosophila funebris* and *Drosophila melanogaster*. Amer. Nat., **85**: 159–69.

Moore, J. A. 1952. Competition between *Drosophila melanogaster* and *Drosophila simulans*. I. Population cage experiments. Evolution, **6**: 407–20.

Nicholson, A. J. 1957. The self-adjustment of population to change. Cold Spring Harbor Symp. Quant. Biol., **22**: 153–73.

NOTES AND COMMENTS

SOME CYTOTAXONOMIC PROBLEMS IN THE CRASSULACEAE

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For some years the author has been engaged in a study of the chromosomes of all available members of the Crassulaceae. Objectives of this survey have been two: first, to appraise the classification of species in this natural, but taxonomically difficult, family and where possible to decide between conflicting arrangements; and second to note the general systems of change in chromosomes that have accompanied evolution in the family and to look for any significant patterns in this. To these ends well over 500 taxa, by a reasonably conservative estimate, have been studied and several problems have become apparent.

Probably the most conspicuous problem is the frequency with which heteroploidy occurs within what many authors have regarded as single species, especially in the genus *Sedum*. Though many of these species are polymorphic and include various numbers of synonyms, indicating that other authors have noted differences, these often do not correspond well with the differences in the chromosomes, and the situation usually remains confused. Obviously any definitive treatment of such complexes requires a careful correlation of the morphological, geographical, and cytological evidence before any reliable taxonomic decision can be made. The present confusion stems from the incompleteness of the information available and from drawing too sweeping conclusions from such fragmentary information.

Baldwin (1942, 1943) has published maps showing the distribution of diploid, tetraploid, and hexaploid races in *Sedum ternatum* ($x = 8$) and *S. pulchellum* ($x = 11$) of the eastern United States. The morphological differences within the species, however, are not all consistently correlated with the differences in ploidy, and the different chromosome races cannot always be reliably identified by their morphology. In the western United States and Canada, *Sedum lanceolatum* ($x = 8$) also has diploid, tetraploid, and hexaploid races, and the related *S. stenopetalum* (often called *S. douglasii*) is typically octoploid ($n = 32$). *S. wrightii* of the southwestern U. S. and adjacent Mexico has diploid through octoploid races based on $x = 12$, and one Texas population with 13 is known. Some other North American species have similar situations but are not yet so well documented. On the other hand, 37 collections of *S. spathulifolium* from southern California to Vancouver Island all have $n = 15$. This seems to be the only widespread species of the United States and Canada that is reasonably constant cytologically (in spite of the fact that it has several fairly distinct subspecies).

In Europe much the same situation seems to prevail, with many of the widespread species having several chromosome races. For example, the common race of *Sedum album* (at least in cultivation on this continent) has $n = 34$ and this number is found in specimens from the Alps. However, Moroccan and Spanish plants (ssp. *gypsicolum*?) had $n = 51$, and one from Portugal had $n = 68$. Clearly the basic number is 17, but no diploid has yet been found. The species, in the broad sense, is highly polymorphic, with a long list of synonyms, and some splitting may eventually be justified. *S. acre*, as broadly conceived, has races with $n = 20$ and $n = 40$ and also is rather confused taxonomically. Other heteroploid European species (in the broad sense) include *S. hispanicum* ($n = 6$, 7, 14, 15, 20, 21), *S. anglicum* ($n = 12$, 23, ca. 60), *S. stribrnyi* ($n = 16$, 32), *S. dasyphyllum* ($n = 14$, 21, 28), the *S. reflexum* complex, and at least three species of *Sempervivum* (*S. arachnoideum*, *S. montanum*, and *S. tectorum*).

Most of the cases cited so far have involved simple polyploidy, but aneuploidy also is known. *S. glaucophyllum* of Virginia and adjacent states has chromosome races with $n = 14$, 22, and 28 (Uhl, unpublished). The 22-chromosome race has the same number of large chromosomes as the 14-chromosome race, and this clearly is not a simple case of polyploidy based on 7. Baldwin (1944), who knew only the 14-chromosome race, suggested that it originated as an amphiploid between something like *S. nevii* ($n = 6$) and diploid *S. ternatum* ($n = 8$). This still seems the best guess. In such case, the 22-chromosome race of *S. glaucophyllum* may be an autoallohexaploid with a double dose of chromosomes from *S. ternatum*.

In Japan and Korea a complex of species related to *S. polytrichoides*, and including *S. coreense*, *S. kiusianum*, and *S. yabeanum*, has a bewildering array of chromosome numbers: 11, 12, 14, 16, every number from 20 to 27, and 35. Several meiotically irregular collections have been found, suggesting that hybrids between the chromosome races occur. Though there is considerable morphological variation here, the cytological variation seems to be much greater.

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The general problem in cases like these is the taxonomic disposition that should be made of the various chromosome races. In many cases the differences in chromosome number are such as clearly to minimize or prevent gene interchange, and this is an important criterion for different species. Where readily recognizable morphological differences can be found and occur consistently, taxonomic recognition is obviously appropriate. However, the morphological differences between the chromosome races sometimes seem negligible or unreliable, and in such cases it does not appear to be a workable solution to anoint them as separate taxonomic species.

Typically the Crassulaceae are denizens of cliffs and rocks. Such habitats usually are discontinuous and, in terms of geologic time, rapidly changing. Populations must fluctuate greatly in size and in degree of isolation as the area of suitable habitat changes with climate and erosion. Such conditions are generally considered to be conducive to rapid evolution. Cytological impediments or barriers to gene interchange, in the form of the various karyotypes, have evolved and have become established and often uniform in certain of the isolated populations. However, in these cases of intraspecific heteroploidy each karyotype has not yet evolved its own distinctive genotype and phenotype, probably because this is a slower process under these conditions. That is, the cytological characteristics that often distinguish different species have evolved faster than the morphological characteristics. The high frequency of heteroploidy within species may indicate that in fact many of these groups are evolving rapidly at the present time.

A second problem that emerges from a cytotaxonomic survey of the Crassulaceae is the difficulty of determining the basic chromosome number in some groups. In view of the extent of intraspecific heteroploidy, it is hardly surprising that there should be great diversity in chromosome number in some genera. Similar cases of the "*Carex*-type" of diversity in chromosome numbers are known in other families, but nowhere is it better developed than here. Incidentally, though the chromosomes in this family are usually small to very small, they appear to have localized kinetochores. The diversity in chromosome number cannot be attributed here, as it has been in *Carex* (Davies, 1956), to the presence of diffuse kinetochores and the resultant fact that all pieces of a broken chromosome can perpetuate themselves.

In the largely Mexican genus *Echeveria* many different chromosome numbers occur; in fact every gametic number from 12 to 34, except 20, as well as higher numbers up to about 260, has been found in the 100 or so taxa so far investigated (Uhl, unpublished). In some cases similar species have the same or similar chromosome numbers, but in other cases they do not. In *Villadia*, a similar situation prevails, with numbers of 10, 11, 12, 13, 15, 17, 20, 21, 23, 25, 33, 44, 49, and 89. Apparently 11 is a basic number here, but the situation is complicated. In the genus *Sedum* every gametic chromosome number from 4 to 38 is known, as well as many higher numbers (mostly unpublished). However, this genus is considered the ancestral one for the family, and it serves as a repository for many short evolutionary lines that do not fit well elsewhere. In such a family its cytological diversity is to be expected.

The significance of these examples of chromosomal diversity is best considered in the light of examples of relative chromosomal stability in the same family. The South African genera *Cotyledon* and *Adromischus*, for example, fairly consistently have 9 large chromosomes, the largest in the family. In *Cotyledon* all 44 collections studied are similar in this respect, but in *Adromischus* some polyploidy is present (Uhl, 1948 and unpublished). In the genus *Dudleya*, though about one-third of the taxa are polyploids they always have exact multiples of the basic 17 (Uhl and Moran, 1953). About 90% of the approximately 80 species of *Crassula* studied have 7 chromosomes or a multiple, with the remainder having 8 or a multiple (Uhl, 1948, and unpublished).

Clearly the pattern of evolutionary chromosomal change has not been the same in all groups of the family. The relative stability of the karyotype and the kinds of change to which it is susceptible must themselves be genetically controlled characters and products of long evolution. In other words, particular patterns of *change* in karyotype are characteristics of particular groups and probably should be regarded as having major phylogenetic significance.

The third cytotaxonomic problem in the Crassulaceae arises from the frequency of very high chromosome numbers in some taxa, principally in Mexico and South America. *Gametic* chromosome numbers higher than 100 are known in at least 16 species (Uhl, unpublished), and at least six of these species have numbers higher than $n = 154$, which is the highest definite number that has been reported previously in Angiosperms (*Morus nigra*, Darlington and LaCour, 1942). The type collection of the Mexican *Graptopetalum macdougallii* has $n = 192$, probably, but another collection, apparently the same species, has $n =$ ca. 244. An undescribed species of *Echeveria* from Peru has about 260 gametic chromosomes, though minor meiotic irregularities have so far prevented an exact count. This is the highest chromosome number yet reported in Angiosperms. In all these cases the chromosomes are very small, but meiosis is fairly regular.

It is probable that the high numbers are due to polyploidy of some sort carried to a very high degree and that they are not the result of fragmentation, for the chromosomes, though very small, are not significantly smaller than those in

many related species with much lower numbers. Since it has now been well established that in related forms the amount of desoxyribonucleic acid (DNA) generally depends upon the chromosome number and is doubled when the number of chromosomes is doubled (Vendrely and Vendrely, 1956), studies, to be undertaken in the future, of the amount of DNA per nucleus in these species should be very interesting and should indicate whether or not they are true polyploids. Indirect evidence favoring polyploidy comes from the field of radiation biology. Dr. Arnold Sparrow, of the Brookhaven National Laboratory, has found (1955) that *Graptopetalum macdougallii*, $n = 192$, can survive extremely high doses of radiation (200,000 r of X-rays). This agrees with a theoretical expectation that a high polyploid, having many replicates of the same general basic set of chromosomes and genes, should be able to tolerate a high dose of radiation.

One is tempted to speculate concerning the mechanism of chromosome pairing at meiosis in these plants. It is hard to believe that each chromosome is able to synapse with only one other among four or five hundred pairing chromosomes. It seems likely in these cases that any particular chromosome has several to many possible synaptic partners and that it actually pairs with the first one of these it encounters during the synaptic stage (zygotene). Because of the short length (small size), pairing begun between two chromosomes is soon completed and probably only one chiasma per bivalent occurs. This means that, though several chromosomes might have the capacity to pair with each other, multivalent synaptic configurations probably are rare and, if formed, do not persist until metaphase of the first meiotic division (the stage studied) because of the lack of enough chiasmata to hold them together. Genetically such a system should resemble an autopolyploid to some extent, but no work along these lines has been done with this material.

Summary

Cytological study of over 500 taxa of the Crassulaceae has revealed several general problems. First, intraspecific heteroploidy is rather frequent, with some species, as generally conceived, having as many as five or more chromosome races that differ by polyploidy and/or aneuploidy. Morphological variation within the species is not always closely correlated with the differences in chromosome number. The frequency of such incipient species suggests rapid evolution. Second, some genera are extremely diverse cytologically, with no one basic or ancestral number evident. Other genera are cytologically more homogeneous. This difference in stability of chromosome number must be genetically controlled and apparently reflects different modes of speciation. Third, extremely high chromosome numbers are rather common, with *gametic* numbers exceeding 150 known in six Mexican and South American species. Chromosomes of these species are very small, but the available evidence suggests they arose as true polyploids and not as a result of fragmentation.

Acknowledgments

Observations and conclusions reported here are based on cytological study of plants obtained from many sources. The author wishes to acknowledge in particular his debt to Professor R. T. Clausen of Cornell, Dr. Reid V. Moran of the Natural History Museum, San Diego, and Mr. P. C. Hutchison of the University of California Botanical Garden for much material, many identifications and the interchange of ideas.

Discussion of several of the groups cited is based on many counts that have not yet been published. The study is continuing and it is hoped to publish the documentation for these counts in the near future.

Literature Cited

Baldwin, J. T. 1942. Polyploidy in *Sedum ternatum* Michx. II. Cytogeography. Amer. J. Bot., **29**: 283–286.

——. 1943. Polyploidy in *Sedum pulchellum*. I. Cytogeography. Bull. Torrey Bot. Club, **70**: 26–33.

——. 1944. Affinities of *Sedum nevii*. Rhodora, **46**: 450–451.

Darlington, C. D., and L. F. LaCour. 1942. The Handling of Chromosomes. New York. Plate IV.

Davies, E. W. 1956. Cytology, evolution and origin of the aneuploid series in the genus *Carex*. Hereditas, **42**: 349–365.

Sparrow, A. H. 1955. A survey of the radiosensitivity of some higher plants. Rad. Res., **3**: 349.

Uhl, C. H. 1948. Cytotaxonomic studies in the subfamilies Crassuloideae, Kalanchoideae, and Cotyledonoideae of the Crassulaceae. Am. J. Bot., **35**: 695–706.

—— and R. V. Moran. 1953. The cytotaxonomy of *Dudleya* and *Hasseanthus*. Am. J. Bot., **40**: 492–502.

Vendrely, R., and C. Vendrely. 1956. The results of cytophotometry in the study of the deoxyribonucleic acid (DNA) content of the nucleus. Internat. Rev. Cytol., **5**: 171–197.

FICUS AND *BLASTOPHAGA*

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Very largely as a result of the observations made by Charles Darwin on pollination systems and the experiments by which he demonstrated at least a part of the value of cross-fertilization for many plants, naturalists in the second half of the Nineteenth Century were stimulated to study flowers and their pollinators in some detail. One outcome of this was the production of long lists of flower visitors for each species of plant although not all of these vectors are equally efficacious in promoting cross-pollination within the boundaries of the species.

In more recent years, a different outlook has prevailed and there has been a much greater awareness of the value to plants in the utilization of highly discriminatory pollen vectors which, by their constancy in visiting the flowers of a particular taxon, avoid waste in the distribution of pollen. It is even possible that we have tended to go too far in believing that specialized flowers are adapted only to visits by a single kind of pollen vector (Baker, 1961). Also, it has been pointed out (Baker, 1960, 1961) that complete inter-dependence between a group of plants and a particular pollinator may have a restrictive effect upon the further evolution of both parties, in contrast to the apparently favorable effect of close but not exclusive mutual adaptation. Some potentiality for gene-flow appears to be desirable to provide for future adaptation.

For this reason, it is pleasing to see that my friend, Dr. L. van der Pijl, in a recent review in this journal, also points to the evolutionary dangers of such a one-to-one relationship (van der Pijl, 1960). Nevertheless, as his excellent paper is likely to become a standard work of reference on the ecological aspects of flower evolution, it is important that we should pay attention to an apparent exception to this evolutionary dictum. Van der Pijl writes, ". . . the genus *Ficus*, each species with its own gall-wasp, is still flourishing." If it be true that the enormous genus *Ficus* has developed to its present world-wide extent despite the handicap of complete monotropism, we are faced with a paradox which cannot be pushed aside lightly.

In making reference to the available literature, however, I have failed to find any first-hand statement of evidence that complete monotropism, on the species level, really exists. Indeed, there are some indications that various species of *Ficus* actually share pollinators, even though the subject cannot be regarded as sufficiently studied for a convincing comprehensive statement either way. Mayer (1882) investigated a number of Old World fig species and, in some cases, found gall-wasps of two genera, *Blastophaga* and *Sycophaga*, together in the syconia. Fritz Müller (according to his brother Hermann Müller, 1883) examined 10 species of *Ficus* in the Brazilian province of Santa Catharina and found "an astonishing variety of wasps belonging to the Agaonidae; some of these belong to the genus *Blastophaga*, some to a genus like *Ichneumon*." In cases like this, however, it would be helpful if the observers made certain that they could distinguish the wasps which are useful as pollen-vectors from those which are merely parasitic.

Eisen (1901), who reports that gallwasps of more than one species, even more than one genus, may inhabit the same species of fig, also points out that some closely related species of *Ficus* may utilize the same species of gallwasp (cf. also, Corner, 1940). McClean and Ivimey-Cook (1956), quoting from an unidentified source, indicate that while some gallwasps are restricted to a single host, others, like *Blastophaga brasiliensis*, may pollinate a number of fig species.

The taxonomy of this huge genus of trees and climbers is so badly confused that estimates of the numbers of species among plants already examined range from about 600 to more than 2,000. In many cases, according to Standley (1917), taxa are very variable and extreme forms may be connected by a range of intermediates. Part of this bewildering array of morphological types may result from the extreme ease of vegetative reproduction in the genus, whereby mutant forms may be propagated and masquerade as separate species when they come to occupy a large enough area.

Related to this is the possibility of agamospermic reproduction by some species, agamospermy being notoriously responsible for the production of taxonomically impossible diversity in such genera as *Hieracium* and *Rubus*. None of the chroniclers of apomixis in the flowering plants (e.g., Gustafsson, 1946–7; Nygren, 1954) mentions agamospermy in the Moraceae — and yet there is an account, referring to *Ficus* itself, buried in the Annals of the Royal Botanical Garden, Calcutta. There, in 1888, an intensive study was made of cultivated trees of *Ficus Roxburghii* Wall. (Cunningham, 1888). Oviposition by gall-wasps was found to be essential for the successful maturation of syconia on the two types of tree (bearing ovulate flowers and gallflowers plus staminate flowers, respectively). However, there was no evidence of adequate pollen transfer to the ovulate flowers (which appeared to develop their embryos by adventitious embryony from the nucellus). The indirect effect of the wasps

in promoting the retention and development of the syconia would, presumably, be hormonal. If this be true, it remains to be explained how the supply of staminate trees is maintained (unless the agamospermy is facultative only), but there is no question that the possibility of apomixis as a factor in the diversification of *Ficus* cannot yet be ruled out. If it should be proved to be operative, it will be interesting on another account—that every species of *Ficus* examined cytologically has shown the same, diploid chromosome number, $2n = 26$.

Another unexplored possibility is that at least some of the intermediate forms may owe their appearance to natural hybridization between taxa. After all, a famous horticultural item is the hybrid between the edible fig (*Ficus carica* L.) and the creeping (or climbing) small-leaved species *Ficus pumila* L. (*F. repens* auctt.) which is frequently grown as a cover for walls. *F. carica* may also be hybridized with more closely related species and it has been suggested that the cultivated figs have a history which involves more than one act of interspecific hybridization (Condit, 1947).

If hybridization between such diverse species as *F. carica* and *F. pumila* is possible, it may be that only the natural eagerness of taxonomists to name new "species" has prevented the recognition of some of the intermediates between extreme forms as hybrids. Hybridization of this sort, if it should occur naturally, would indicate that pollinating wasps do not restrict themselves without exception to a single species of fig. On the other hand, should a comparison of experimental hybrids with naturally occurring plants show that hybridization is genetically feasible but does not occur, then the restrictive effect of host-preference by the wasps would receive support.

Much biosystematic work on this important genus is needed and the places where it should be done are in the tropics. In truth, it is only for a handful of the legions of species that the relation between the tree and the wasp is known in any detail. In particular, we know nothing at all of the pollination mechanisms of the species which produce syconia below ground. Nevertheless, there is hope that one unique feature of this genus may enable interested entomologists to make a significant contribution to our knowledge. Thus, any herbarium specimens bearing immature syconia stand a good chance of containing dried remains of the pollinating insects within them, especially if the syconia are such as bear the gall-flowers. They may be dissected to provide material for identification of the wasps.

Literature Cited

Baker, H. G. 1960. Reproductive methods as factors in speciation in flowering plants. Cold Spr. Harb. Symp. Quant. Biol., **24**: 177–191.

——. 1961. The adaptation of flowering plants to nocturnal and crepuscular pollinators. Quart. Rev. Biol., **36**: 64–73.

Condit, I. J. 1947. The Fig. Waltham, Mass.

Cunningham, D. D. 1888. On the phenomenon of fertilization in *Ficus Roxburghii* Wall. Ann. Roy. Bot. Gard. Calcutta, **1** (Appendix): 11–47.

Corner, E. J. H. 1940. The Wayside Trees of Malaya, vol. 1. Singapore.

Eisen, G. 1901. The Fig; its History, Culture and Curing. U.S.D.A., Div. Pomology Bull. no. 9. Washington.

Gustafsson, A. 1946–47. Apomixis in higher plants. I–III. Lunds Univ. Årsskr. N. F. Avid. II, **42**(3); **43**(2); **43**(12).

Mayer, P. 1882. Zur Naturgeschichte des Feigeninsekten. Mitt. aus d. Zool. Stat. Neapel., **3**: 551–578.

McClean, R. C., and W. R. Ivimey-Cook. 1956. Textbook of Theoretical Botany, vol. II. London.

Müller, H. 1883. The Fertilisation of Flowers (trans. and ed. by D'Arcy W. Thompson). London.

Nygren, A. 1954. Apomixis in the Angiosperms. II. Bot. Rev., **20**: 577–649.

Standley, P. C. 1917. The Mexican and Central American species of *Ficus*. Contr. U. S. Nat. Herb., **20**: 1–35.

van der Pijl, L. 1960. Ecological aspects of flower evolution. I. Phyletic evolution. Evolution, **14**: 403–416.

LETHALS IN ONTOGENY

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Professor Hadorn must look upon the English text of his book[1] with mixed feelings. He may well be gratified by the complimentary reception the German version had when it appeared in 1955 and by the resulting decision to translate it; but to publish essentially the same book in 1961 involved, as he says, the serious handicap of having to ignore the literature of the last four or five years. A reader will find himself valuing the book as a source of historical information rather than for the author's views on current problems. Time confirms the earlier reviewers' conclusions that Hadorn has produced a comprehensive scholarly work, and in the six years that followed its appearance nothing comparable or competitive has been published. The early chapters describe the behavior patterns of genes and illustrate them with great thoroughness and numerous references and examples all chosen from the special if ill-defined category of lethals. There follows a rather larger part dealing with developmental problems such as pleiotropy, cellular autonomy, and phenocopies. Since, quite properly, considerable use has been made of the author's own work, this part provides a very interesting and readable account of his experiments and his views. Perhaps these later chapters of the book are those which have best retained their value and freshness.

The title as translated has an ambiguity that provokes some comment. If the book was intended to be primarily about lethals, then a reader could reasonably expect rather less discussion of development and rather more of radiation-induced lethals and of lethals viewed from the angle of population genetics. Recent work on the fertilization, division, and implantation of mammalian eggs has had to be left out of consideration, but the resulting weakness in the treatment of maternal effects and the causes of death in early embryonic life is apparent. Since in fact the book is intended to be primarily about developmental genetics in the light of the misdevelopment of zygotes carrying lethals, then some of the supporting material detracts from the main purpose and is occasionally even misleading. There is a hint of unreality about "lethal factors *sensu stricto* are characterised by a penetrance of 100%" followed by descriptions of various other categories of factors (e.g., subvitals) which for one reason or another do not make the grade. From the author's own point of view when he came to developmental processes, the making of categories must have been an embarrassment.

By definition a lethal kills before reproductive age is reached. If failure to transmit genes to the next generation is the important idea, then there is no worthwhile distinction between lethals and sterility genes. If a lethal is to be regarded as an opportunity for the study of development, then the ultimate death is barely relevant. Hadorn does not in fact make much of the fact of death except in the first few chapters where it is manipulatively convenient, and in chapter 19 where it is economically inconvenient. He views the activities of his factors in the context of varying genotype and environment and does not expect them to function in a formal Mendelian manner at all times. He is essentially dynamic in outlook. Any early static attitudes were doubtless adopted for didactic purposes—which may also account for the somewhat outmoded word "factor."

The chapters on developmental genetics are all good reading, especially those which deal with pleiotropy and phenocopies. A phenocopy is an environmentally produced alteration in the manifestation of a genotype which copies the pattern of manifestation of another genotype and uses the same developmental pathways at least to some extent. During the last few years, the opportunities inherent in this field of study have considerably expanded and much further expansion seems imminent. As Hadorn says, the combinations of environmental factors and genotypes are legion, and only a relatively few have been tried. In mammals gestation offers a sensitive period the importance of which to subsequent growth and development is slowly being appreciated. Since technical skills are growing, especially in biochemical assay and surgery, the study of phenocopies in large animals is likely to flourish increasingly and Hadorn's account of them will be a help to those interested in learning about this field.

Hadorn was a pioneer in the study of biochemical variation and here the growth of knowledge in recent years has been so fast that it is most regrettable he was unable to use it. Eventually the description of developmental pathways will have to be in biochemical rather than morphological terms, and consequently the recent advances in the methodology of searching for biochemical variants bode well for this purpose. Hadorn points out in his chapter on the possibilities of therapy that there may be no reason to hope for wholesale curative measures to counteract the action of deleterious genes; but a deeper understanding of the fundamental biochemical steps in develop-

[1] Hadorn, E. 1961. Developmental Genetics and Lethal Factors. xviii, 355 pp. 129 figs. Translated by Ursula Mittwoch. $8.50. Methuen (London) and Wiley (N.Y.).

ment seems to be well on its way and promises at least some success in producing what might be called controlled phenocopies of the better adapted phenotypes.

One wonders why it was felt necessary to include chapter 19 on economic losses among domestic animals. It is a pity that when he comes to deal with animals of economic importance, Hadorn casts aside his normal scientific standards. For instance, he solemnly repeats an improbable anecdote that should never have appeared in the original paper (about hemophilia in pigs); and he borrows a diagrammatic pedigree purporting to show the heredity of *atresia coli* in the horse (1928) although his students would scoff at any modern author who selected his data in the manner shown. Economic losses are not in fact easy to assess, and a gratuitous assumption of awe at the financial aspect of lethals can lead to error. For years geneticists have been saying, as does Hadorn, that the way to deal with lethals in livestock is to make test-crosses. Yet when the need is apparently greatest, as in the practice of artificial insemination of cattle, the tests are not made —and for good reasons. Even if the operators of artificial insemination services knew what particular lethal to test for, the cost of test-crossing for all bulls would be economically more serious than to spread the lethal. An interesting problem will arise some day when a really good animal (as distinct from an old-fashioned show winner) capable of transmitting much improved performance is recognized to be transmitting a lethal or other deleterious gene as well.

One of the most interesting aspects of lethals in livestock is not mentioned. Recognizable lethals are far from common, and in practical livestock breeding of cattle, sheep, and pigs, they cause very little trouble. Inbreeding decreases fertility and increases the incidence of defectives of vague genetic origin, but good Mendelian lethals are rarely seen to occur. In the large and complex animals whose foetal stages are carried out in expensive seclusion, lethals may usually act too soon to be seen—except as reduced fertility.

The translation has been well done and the book is very lucid and readable.

BIOLOGICAL CLOCKS

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If one were called upon to name an area within the subject of animal behavior which is expanding with the kind of explosive rapidity which we usually associate with atomic physics, none would be more appropriate than the study of the mechanisms of celestial orientation in animals, and the time sense with which they are often associated. Since the war, workers on both sides of the Atlantic have focused a major research effort upon these problems. The organizers of the Twenty-fifth Cold Spring Harbor Symposium on Quantitative Biology, under the chairmanship of C. S. Pittendrigh, succeeded magnificently in assembling a truly international array of authorities to speak in June 1960 on the subject of "*Biological Clocks.*"[1]

A proportion of the papers was devoted to establishing the presence of periodicities in the behavior of animals, some upon a lunar or annual time scale (Fingerman, Hauenschild, Marshall, Wolfson), others upon a diurnal scale. Plants were also considered, particularly in the section on thermo- and photoperiodism, establishing that similar periodic phenomena occur here and in animals (Bünning, Bünsow, Hendricks, Highkin, Lees, Hamner, Schwemmle, Went, Wilkins). This interdisciplinary approach was a major factor in the advances made by the conference. It was well personified in the erudite opening address by Bünning, calling equally freely upon examples from periodic leaf movements in bean plants and activity rhythms in cockroaches. Nor was the ecological aspect of these phenomena entirely neglected (Cloudsley-Thompson, Corbet, Renner).

Most attention was given to diurnal periodicities, defined here as "circadian" rhythms because the period usually only approximates 24 hours, which prove to be amazingly widespread and perhaps "ubiquitous in living systems." Pittendrigh and Aschoff review general properties of rhythms in masterly fashion, pointing out those characteristics which are already established and suggesting areas for future research. With one important dissent (Brown) it is generally accepted that such rhythms usually have an endogenous basis. Nevertheless they are subject to various kinds of influence by external stimuli. Environmental "perturbations" such as a change in light–dark rhythm, a flash of light or a shift in the cycle of temperature change may establish a new phase relationship without affecting the period length. Moreover the extent of resetting varies according to the phase of the existing cycle in which the perturbation occurs (Aschoff, Bruce, DeCoursey, Renner), a point of special interest to comparative psychologists since it has much in common with variations in the motivation of other types of behavior. Such external stimuli as light intensity may also modify the period length, the direction of change being related to the habits of the animal. Thus an increase in the intensity of a continuous light results in a longer period in nocturnal animals, and a shorter one in diurnal animals (Aschoff). The relative constancy of circadian rhythms over wide ranges of temperature, a prerequisite for any clock mechanism, presents a special problem to the physiologist (Sweeney, Hastings, Rawson, Ehret), and Sweeney and Hastings even suggest that "it is the most important single factor in sparking the interest of scientists in the problem of biological rhythms." The next few years will probably see a surge of interest in the physiological mechanisms which are involved here, and the papers included in the symposium will serve as an effective prelude (Halberg, Hellbrügge, Lobban, Mori), particularly the elegant experiments of Harker on the nervous and endocrine factors controlling circadian rhythms in the cockroach. Closely linked with these analyses are the various types of theoretical models which have been set up to detect periodic phenomena (Mercer) and to imitate the kind of oscillating systems which these behavioral rhythms represent. Once again the organizers are to be complimented on including engineers as well as biologists on the program, the ensuing discussions serving to clarify the advantages and dangers of various types of models in a unique way (Barlow, Ehret, Kalmus, Klotter, Pittendrigh, Schmitt, Wever, Wigglesworth). For some readers the climax of the book will be the eleven papers in which the time sense is considered in relation to a specific behavioral problem, namely the use of celestial cues in animal orientation. The ability to orient to the sun, moon, stars, and patterns of polarization in the daytime sky and to allow for their movement during the course of the day is carefully documented and analyzed for birds (Hoffmann, Sauer, Schmidt-Koenig, Wallraff), fish (Braemer, Hasler, Schwassmann), and arthropods (Birukow, Lindauer, Papi, Pardi). There is no more up-to-date review available of the subject of animal orientation and navigation than that given in this series of papers, which comes right up to the present limits of our knowledge.

If this book simply consisted of a review of material available elsewhere it would be of great value, for the time interval between the conference and publication has been remarkably short. In fact it is something more than that, containing as it does the kind of new ideas which arise only when workers from diverse disciplines bring their talents to bear upon a common problem. It may be read with interest and enlightenment by zoologists, botanists, ecologists, physiologists, behaviorists, and psychologists and perhaps even by engineers. What more could one ask?

[1] Cold Spring Harbor Symposia on Quantitative Biology, vol. 25, xiii, 524 pp. 1961. $8.00.

BOOKS RECEIVED

Notices will be given under this heading of books of evolutionary interest sent to the Editors. Appearance of a title in this section will not preclude a fuller review in a later issue of the journal.

Müntzing, A. 1961. Genetic Research. 345 pp. 213 figs. LTs Förlag, Stockholm (36 Swed. Cr.). An English translation by the author of the second Swedish edition of the textbook on general genetics.

Salton, Milton R. J. 1960. Microbial Cell Walls. IX + 94 pp. 20 figs. $3.50. Wiley, New York. The fourth volume of the CIBA lectures in microbial biochemistry presented at Rutgers University.

Cain, A. J. (ed.). 1959. Function and Taxonomic Importance. 140 pp. illus. $2.00. Systematics Association (British Museum, Cromwell Road, London S. W. 7, England). A collection of papers presented at a symposium of the Systematics Association. The first, theoretical, part contains a review by the editor of the historical controversy regarding the importance of function as a taxonomic criterion and a brief note by Julian Huxley on certain terminological aspects of evolution. The second part contains a group of papers dealing with the application of functional analysis to certain arthropods (S. M. Manton), wallabies (W. D. L. Ride) and sea-urchins (D. Nichols). The third part comprises articles illustrating difficulties of the application of function analysis to taxonomy. It includes contributions on environmental modification of taxonomic traits (G. Ainsworth Harrison), on ecotypic variation of plants (V. H. Heywood), on classification of turacos, an African family of birds, (R. E. Moreau), on speciation in charrs, a salmonid fish, (G. F. Friend), and, finally, on behavioral characteristics as a taxonomic criterion (J. M. Cullen).

Fitch, Henry S. 1960. Autecology of the Copperhead. Univ. Kans. Publ. Mus. Nat. Hist., 13(4):.85–288, 8 plates, 26 figs.

Wilson, R. W. 1960. Early Miocene Rodents and Insectivores from Northeastern Colorado. Univ. Kans. Paleont. Contr., Article 7. 92 pp. 131 figs.

Eaton, T. H., Jr. 1960. A New Armored Dinosaur from the Cretaceous of Kansas. Univ. Kans. Paleont. Contr., Article 8. 24 pp. 21 figs.

Terentjev, P. V. (ed.). 1960. Application of Mathematical Methods in Biology (title translated). 227 pp. illus. 0.98 New roubles. Leningrad University. Leningrad. Proceedings of a conference on biometry held at the University of Leningrad in 1959. Papers of evolutionary interest by the editor, R. L. Berg, I. I. Schmalhausen and others are included. A table of contents in English, but no summaries, is provided.

ANNOUNCEMENT

The Permanent International Committee for Genetics Congresses has accepted the invitation of the Biological Council of the Royal Netherlands Academy of Sciences to hold the 11th International Genetics Congress at The Hague-Scheveningen, Netherlands, provisionally on September 2–12, 1963. Professor C. L. Rümke is the President of the Executive Committee of the Congress and Professor S. J. Geerts (Genetisch Laboratorium, Driehuizerweg 200, Nijmegen) is the Secretary.

INFORMATION

Contributions to systematic biology from the fields of [illegible], taxonomy, paleontology, [illegible] biology, provided that the [illegible] the student of evolution. [illegible] basis of the evolutionary [illegible] members of the Editorial Board or other competent [illegible]

The length of manuscripts will [illegible] to twenty printed pages. [illegible] is granted by the Editorial Board. [illegible] in a separate section, "Notes and Comments." [illegible] have to be kept within economic limits.

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Authors are requested to prepare abstracts of their papers to be returned with the proof to the Managing Editor to be forwarded to Biological Abstracts.

Manuscripts should be addressed to

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